Interleukin-6 (CLIA) PACKAGE INSERT

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INTENDED USE

Interleukin-6 (CLIA) is a Chemiluminescence Immunoassay test for the quantitative measurement of circulating interleukin-6 (IL-6) in human serum, plasma and whole blood (Heparin sodium/Heparin lithium/EDTA) ,which is intended as an aid to assess to monitor the immune state and inflammatory response of the body
The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120, and all other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of functions. IL-6 is produced from a single gene encoding a product of 212 amino acids, which is cleaved at the N terminus to produce a 184 amino acid peptide with a molecular weight between 22-27 kDa.[1]
In vitro, IL-6 is produced by a variety of cell types such as T lymphocytes, B cells, macrophages, eosinophils, epithelial cells, and fibroblasts. IL-6 production is rapidly induced in the course of acute inflammatory reactions associated with injury, trauma, stress, infection, brain death, neoplasia, and other situations.[2-4] IL-6 concentrations in trauma patients may predict later complications from additional surgical stress or indicate missed injuries or complications. Sequential measurements of IL-6 in serum or plasma of patients admitted to the ICU (intensive care unit) showed to be useful in evaluating the severity of SIRS (systemic inflammatory response syndrome), sepsis and septic shock and to predict the outcome of these patients. IL-6 is also useful as an early alarm marker for the detection of neonatal sepsis. IL-6 also plays a role in chronic inflammation e. g. rheumatoid arthritis (RA).[2-5]

ASSAY PRINCIPLES

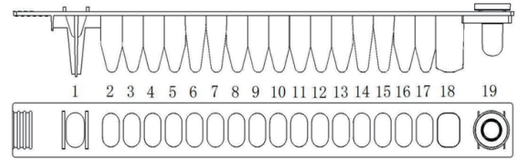
IInterleukin-6 (CLIA) hereinafter also referred to as the "IL-6" is a one-step sandwich assay to determine the level of interleukin-6 (CLIA).
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridge. All the assay steps are performed automatically by the ACCRE analyzer.
The sample is manually pipetted into the sample well of reagent cartridge, then sample, IL-6 antibody labeled with alkaline phosphatase and IL-6 antibody coated magnetic particles are mixed thoroughly together, incubating at 37 °C. The analyte in the sample, labelled antibody and antibody coated with magnetic particles are bonded together as sandwich immunocomplex. The immunocomplex is then washed in the magnetic field to eliminate the unbound components in the sample. The substrate is added to trigger the chemiluminescent reaction, the result of chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). At the end, the results are automatically calculated by the instrument in relation to the calibration curve stored.

CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls.

Contents	Quantities				Notice
	REF W90A	REF W90C	REF W142A	REF W142C	
IL-6 reagent cartridge	60 strips	36 strips	60 strips	36 strips	Ready to Use
IL-6 Calibrator 1 (CAL1)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, 50 mM; ProClin300, 0.48 g/L; BND, 0.2g/L BSA,10g/L;
IL-6 Calibrator 2 (CAL2)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, 50 mM; ProClin300, 0.48 g/L; BND, 0.2g/L; BSA,10g/L; recombinant interleukin-6, 10 pg/mL
IL-6 Calibrator 3 (CAL3)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, 50 mM; ProClin300, 0.48 g/L; BND, 0.2g/L; BSA,10g/L; recombinant interleukin-6, 500 pg/mL
IL-6 Quality Control Level 1 (QC1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Tris buffer, 50 mM; ProClin300, 0.48 g/L; BND, 0.2g/L; BSA,10g/L; recombinant interleukin-6, 10 pg/mL Only for the configuration with Quality Controls
IL-6 Quality Control Level 2(QC2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Tris buffer, 50 mM; ProClin300, 0.48 g/L; BND, 0.2g/L; BSA,10g/L; recombinant interleukin-6, 500 pg/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configuration with Quality Controls

The Reagents Cartridge
The strip consists of 19 wells. The 1st well is the sample well and the last 19th well is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a labeled, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridges. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
3	Sample Treatment Solution	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
5	Enzyme Label	ALP (alkaline phosphatase) labeled anti-IL-6 antibody (mouse), 3.0mg/L; MES Buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
7	Magnetic Particles	Anti-IL-6 antibody (mouse) coated magnetic particles, 0.2g/L; Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
10,11,12,14	Washing Solutions	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	350μL
16	Substrate Solution	Disodium [(4-chlorophenyl)sulfanyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate	100μL

Note: Well 1, 2, 4, 6, 8, 9, 13, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc).
- Pipette with disposable tip to dispense 200 μL.
- Powderless and disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- 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.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Interleukin-6 (CLIA) **upright** at 2-8 °C, kit can be stored for 18 months from manufactured date . After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be completed within 2 hours.
- Store the calibrators and quality controls at 2-8 °C till expiry date. When opened, the calibrators and/or controls will be stable for 45 days at 2-8 °C.
- Keep away from sunlight.
- Do not freeze reagents cartridge.

SAMPLES

The sample volume for Interleukin-6 (CLIA) assay is 50μL, please note that at least 75μL of sample has to be pipetted manually into the reagent cartridge.
Sample type and collection
Human serum or plasma/whole blood (Heparin sodium/Heparin lithium/EDTA).
Types of tubes:
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin sodium,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation
The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples (hemoglobin >1000mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability
Samples (serum and plasma) can be stored at room temperature(15-30°C) for up to 2 hours or at 2-8 °C for up to 24 hours; if longer storage is required, freeze the serum or plasma at -20 °C or below -20 °C for up to 6 months, without exceeding 1 freeze/thaw cycle.
Whole blood samples can be stored at room temperature for up to 2 hours or at 2-8 °C for up to 24 hours and not undergo freeze/thaw cycles.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.
When using the assay for the first time.
Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents: Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration
Calibration must be performed with the reagents and calibrators from Manufacturer.
Calibration is necessary under the following conditions:
(1) New reagent lot number applied;
(2) Same lot of reagents used more than 4 weeks;

(3) Quality control out of control;
(4) When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1. Remove the kit from storage at 2-8 °C and take out the required number of reagent cartridges. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay strips for each sample, calibrator or control respectively.
3. Place the reagent cartridges into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Control into the sample well of each reagent cartridge .

Please note that at least 75μL sample has to be pipetted manually into the reagent cartridge and 50μL sample is needed for each test.

** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent（if applicable) DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. Interleukin-6 (CLIA) assay will be completed within approximately 25 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Traceability

This method has been standardized against the NIBSC (National Institute for Biological Standards and Control) 1st IS 89/548 Standard.

Dilution

Samples with concentrations above 5000 pg/mL can be diluted with **diluent negative serum or plasma** manually. The recommended dilution is 1:10. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples. The concentration of the diluted sample must be >500 pg/mL.

Quality Control

Quality control should be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in pg/mL.

Interpretation of Results

Results of IL-6 assays were determined by testing specimens from a study in clinical centers with group of totally 248 healthy adult individuals in China. The 95th percentile value is 7 pg/mL.

** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range*.

LIMITATIONS

1. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
2. Please note that heterophile antibody in human serum or plasma can interfere the test result.
3. High concentration of HAMA can also cause a false positive or false negative result.
4. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
5. The detection range of the kit is 1.5 pg/mL ~ 5000 pg/mL. Samples with IL-6 concentration below upper detection limit can be quantified , if the concentration is above the upper detection limit, the results are reported as>5000 pg/mL. The product supports manual sample dilution of 1:10 and can be diluted with low-value serum or sample diluent.

PERFORMANCE CHARACTERISTICS

Lower limit of measurement

LoD (Limit of Detection) =1.5 pg/mL

The lower detection limit represents the lowest measurable 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 = 20).

The Limit of Detection was determined in accordance with the CLSI® (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).

The measurement range of the Interleukin-6 (CLIA) assay is 1.5pg/mL~5000 pg/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Interleukin-6 (CLIA) assay is between 1.5pg/mL~5000pg/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

The precision study was performed according to CLSI® EP05-A3 recommendations.
The within-lot precision CV% is less than 8%.
The between-lot precision CV% is less than 10%.

Interfering Substances

Potential interference substances were studied according to CLSI® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤ 1000 mg/dL
Bilirubin	≤ 40 mg/dL
Triglyceride	≤ 3000 mg/dL
Total Protein	≤ 10 g/dL
Rheumatoid factor	≤ 1500 IU/mL

Hook effect

No hook effect was found up to IL-6 concentrations of 200000 pg/mL.

Accuracy

The relative deviation between the measured value and the calibration concentration is within the range of ±10%.

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

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