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Interleukin-8 human ELISA

CE IVD

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Σ **96**

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CONTENTS

1. INTENDED USE

Immunoenzymetric assay for the in vitro quantitative measurement of human interleukin-8 (IL-8) in plasma.

2. CLINICAL BACKGROUND

A. Biological activities

IL-8 (also known as NAP-1 for Neutrophil-activating peptide) is a chemoattractant protein for neutrophils. This cytokine belongs to a new family of chemotactic peptides called "chemokines". This proinflammatory mediator is secreted by different cells such as monocytes, neutrophils, endothelial cells, fibroblast after activation, and by mitogen-stimulated T lymphocytes. IL-8 is a key cytokine that has been found in scales of psoriasis patients, in synovial fluid of patients suffering from rheumatoid arthritis and gout. The role of IL-8 in the recruitment of neutrophils in the lung during ARDS has also been suggested.

B. Clinical application

The IL-8 level in the septic shock patients was found to correlate with mortality and in acute graft liver rejection the IL-8 serum levels were reported to have markedly increased. The level of IL-8 in these or other conditions may prove to be important in characterizing the progress of these disease conditions.

3. PRINCIPLES OF THE METHOD

The Demeditec IL-8-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-8. Calibrators and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human IL-8 – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the IL-8 concentration. A calibration curve is plotted and IL-8 concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration range.

4. REAGENTS PROVIDED

Reagents	96 tests Kit	Reconstitution
SORB MT Microtiterplate with 96 anti-IL-8 (monoclonal antibodies) coated breakable wells	12 x 8 wells	**Ready** for use
ENZ CONJ Conjugate: HRP labelled anti-IL-8 (monoclonal antibodies) in TRIS-Maleate buffer with bovine serum albumin and thymol	1 vial 6 ml	**Ready** for use
CAL 0 – 5 LYO Calibrator 0 to 5 (see exact values on QC data sheet) in human plasma with benzamidin and thymol	6 vials lyophil.	**Add** 1 ml distilled water
SAM DIL LYO Specimen Diluent: human plasma with benzamidin and thymol	2 vials lyophil.	**Add** distilled water (see on the QC data sheet for the exact volume)
INC BUF Incubation Buffer: Phosphate buffer with bovine serum albumin and thymol	1 vial 11 ml	**Ready** for use
WASH SOLN 200x Wash Solution (Tris-HCl)	1 vial 10 ml	**Dilute** 200x with distilled water (use a magnetic stirrer).
CONTROL 1 & 2 LYO Controls 1 and 2 in human plasma with thymol	2 vials lyophil.	**Add** 1 ml distilled water
SUB TMB Chromogen TMB (Tetramethylbenzydine)	1 vial 12 ml	**Ready** for use
STOP SOLN Stop Solution: HCl 1.0N	1 vial 12 ml	**Ready** for use

Note: 1. Use Specimen Diluent for sample dilutions.
2. 1 pg of the calibrator preparation is equivalent to 1 mU of the NIBSC 1st IS 89/520.

5. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 1 ml and 10 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
6. Washer for Microtiterplates
7. Microtiterplate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (bichromatic reading)

6. REAGENT PREPARATION

A. Calibrators: Reconstitute calibrators with 1 ml distilled water.
B. Controls: Reconstitute the controls with 1 ml distilled water.
C. Specimen Diluent: Reconstitute specimen diluent to the volume specified on the QC data sheet with distilled water
D. Working Wash solution: Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

7. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators, controls and Specimen Diluent are stable for 4 days at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 2 months. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at 18-25°C until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

8. SPECIMEN COLLECTION AND PREPARATION

- Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at 18-25°C. It is recommended to vortex the samples before use.
- Sampling conditions can affect values, therefore, strict precautions have to be taken during sampling to avoid impurities contained in sampling materials that would stimulate IL-8 production by blood cells and thus falsely increase plasma IL-8 values.
- Collection tubes must be pyrogen-free. Plasma can be collected on sterile EDTA and rapidly separated after centrifugation. The use of heparin tubes is discouraged as batches of heparin are often contaminated with pyrogen.

9. PROCEDURE

A. Handling notes

- Do not use the kit or components beyond expiry date.
- Do not mix materials from different kit lots.
- Bring all the reagents to 18-25°C prior to use.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
- Use a clean plastic container to prepare the Wash Solution.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.
- High precision pipettes or automated pipetting equipment will improve the precision.
- Respect the incubation times.
- To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in section 12 paragraph E (Time delay).
- Prepare a calibration curve for each run, do not use data from previous runs.
- The Chromogenic Solution should be colourless. If a blue colour develops within a few minutes after preparation, this indicates that the reagent is unusable, and must be discarded.
- Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.
- During incubation with Chromogenic Solution, avoid direct sunlight on the microtiterplate.

B. Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 100 µl of Incubation Buffer into all the wells
4. Pipette 100 µl of each Calibrator, Control and Sample into the appropriate wells.
5. Pipette 50 µl of anti-IL-8-HRP conjugate into all the wells.
6. Incubate for 2 hours at 18-25°C on a horizontal shaker set at 700 rpm ± 100 rpm.
7. Aspirate the liquid from each well.
8. Wash the plate 3 times by:
   - Dispensing 0.4 ml of Wash Solution into each well
   - Aspirating the content of each well
9. Pipette 100 µl of the Chromogenic Solution into each well within 15 minutes following the washing step.
10. Incubate the microtiterplate for 15 minutes at 18-25°C on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
11. Pipette 100 µl of Stop solution into each well.
12. Read the absorbencies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 30 minutes and calculate the results as described in section 10.

10. CALCULATION OF RESULTS

A. Polychromatic Reading:

1. In this case, the software will do the data processing.
2. The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
3. A second reading is performed at 490 nm against the same reference filter.
4. The Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
5. The principle of polychromatic data processing is as follows:
   - Xi = OD at 450 nm
   - Yi = OD at 490 nm
   - Using a standard unweighted linear regression, the parameters A & B are calculated: Y = A*X + B
   - If Xi < 3 OD units, then X calculated = Xi
   - If Xi > 3 OD units, then X calculated = (Yi-B)/A
   - A 4-parameter logistic curve fitting is used to build up the calibration curve.
   - The IL-8 concentration in samples is determined by interpolation on the calibration curve.

B. Bichromatic Reading

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of IL-8 (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

11. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

IL-8-ELISA		OD units Polychromatic model
Calibrator	0 pg/ml	0.029
	40.4 pg/ml	0.120
	58 pg/ml	0.164
	156 pg/ml	0.423
	551 pg/ml	1.350
	1845 pg/ml	2.973

12. PERFORMANCE AND LIMITATIONS

A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average OD at zero binding, was 1,1 pg/ml.

B. Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, TNF-α, TNF-β, IFN-α, IFN-β, IFN-γ, TGF-β, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, RANTES, PF-4, βTG, GRO, IP-10 and SCF. This IL-8 assay is specific for human natural and recombinant IL-8 and is able to recognize the 72 a.a. form of IL-8.

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	**N**	**<X> ± SD (pg/ml)**	**CV (%)**	**Serum**	**N**	**<X> ± SD (pg/ml)**	**CV (%)**
A	12	102 ± 3	3.2	A	20	150 ± 13	8.6
B	12	227 ± 8	3.6	B	20	442 ± 58	13.1

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Sample	Added IL-8 (pg/ml)	Recovered IL-8 (pg/ml)	Recovery (%)
Plasma	0	0	-
	61	65	105
	108	127	118
	292	349	119

DILUTION TEST

Sample	Dilution	Theoretical Conc. (pg/ml)	Measured Conc. (pg/ml)
Plasma	1/1	-	678
	1/2	339	272
	1/4	169	148
	1/8	85	82
	1/16	42	40

Samples were diluted with Specimen Diluent.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrators have been added to the coated wells.

TIME DELAY

sam-ple	0 min	10 min	20 min	30 min	40 min
1	66	53	59	55	62
2	118	114	111	108	113
3	246	224	221	213	221
4	914	906	905	882	855

F. Hook effect

A sample spiked with IL-8 up to 0.5 µg/ml gives higher OD's than the last calibrator point.

13. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the QC data sheet, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls that contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

14. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.
For guidance, the results of 36 EDTA plasma samples from apparently healthy persons with low CRP levels, ranged between 0 and 132 pg/ml. Among them, 34 samples obtained values below 50 pg/ml.

15. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.
The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with all reagents, Stop Solution contains HCl. In case of contact, wash thoroughly with water.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

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SUMMARY OF THE PROTOCOL

	CALIBRATORS (µl)	**SAMPLE(S) CONTROLS (µl)**
Incubation Buffer	100	100
Calibrators (0-5)	100	-
Samples, Controls	-	100
Anti-IL-8 -HRP conjugate	50	50
Incubate for 2 hours at 18-25°C with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Chromogenic Solution	100	100
Incubate for 15 min at 18-25°C with continuous shaking at 700 rpm.		
Stop Solution	100	100
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (and 490 nm) versus 630 (or 650 nm)		

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta