IMMULITE® 2000

ECP

DPC®

IMMULITE® 2000 ECP

Intended Use: *For research use only* with the IMMULITE 2000 Analyzer. *Not for use in diagnostic procedures.*

Catalog Number: **L2KEO2** (200 tests)

Test Code: **ECP** Color: **Aqua**

CDC Analyte Identifier Code: 1616
CDC Test System Identifier Code: 10418
CLIA Complexity Category: Moderate

Summary and Explanation

Eosinophils, originating from bone-marrow stem cells, appear in large numbers at inflammation sites and in response to certain parasitic infections. These leukocytes, when mature, reside mostly in tissues, but about 1% of the eosinophil population circulates in the blood. Activated eosinophils degranulate to release four highly basic proteins into the surrounding tissue. The granular proteins, which can kill parasites and some mammalian cells, might cause the tissue damage associated with asthma and other inflammatory diseases. Eosinophil activation accompanies a wide range of inflammatory conditions, including bronchial asthma, atopic dermatitis, rhinitis, allergic eye inflammation, allergic middle ear effusion, parasitic and bacterial infections, autoimmune diseases, and chronic fatigue syndrome.[11]

Among the four basic granule proteins, eosinophil cationic protein (ECP) has proven a useful monitor for many active inflammatory diseases.[11] ECP concentrations in plasma and certain other body fluids increase during inflammatory reactions marked by activated eosinophils. Produced by eosinophils exclusively, ECP is toxic to neurons, some epithelial cell lines, and isolated myocardial cells. The positively charged protein binds to heparin and inhibits blood coagulation.

Several studies report high individual and group correlations between ECP levels and clinical asthma symptoms, such as increases in peak expiratory flow (PEF), prn ("as needed") inhaled β_2-agonist, airway responsiveness, and spirometry.[1,10] Atopic serum samples have higher ECP levels than nonatopic control samples, even when the circulating eosinophil count remains within the normal range.[9] In seasonal asthmatic patients, ECP measurements reflect changes in disease activity throughout the year.[1,9] Roquet, et al. reported significant correlations between ECP levels and bronchial hyperreactivity in mildly asthmatic patients.[6] Tomassini, et al. showed that serum ECP concentrations exceed normal, control levels in both IgE-mediated and non-IgE-mediated atopic conditions. Serum ECP measurements avoid inconsistencies inherent in subjective asthma assessments.

Serum ECP concentrations can indicate the severity of certain skin disorders.[2,3] ECP's neuronal toxicity might contribute to itching disorders; patients with certain skin disorders, such as papular erythematous eruptions and prurigo nodularis, displayed increased serum ECP levels, which normalized when the conditions healed.[8,3] Several groups found that serum ECP concentrations reflect atopic dermatitis (AD) activity.[2,3] The commonly used clinical scoring system for atopic dermatitis records lichenification, loss of sleep, erythema, papules, pruritus, and excoriations. Czech, et al. showed that ECP correlates with each of these symptoms and most highly correlates with the total clinical score. Although altered immunological parameters accompany atopic dermatitis, measured serum IgE concentrations did not correlate with some of the clinical symptoms.[2]

Principle of the Procedure

IMMULITE 2000 ECP is a solid-phase, two-site chemiluminescent immunometric assay.

Incubation Cycles: 1 × 30 minutes.

Specimen Collection

Collect blood by venipuncture[13] into **Becton Dickinson SST vacutainer tubes**, noting the time of collection. Fill collection tubes completely, gently invert, and let the blood clot for 60–120 minutes at room temperature (15–28°C). Centrifuge at 1,000–1,300xg for 10 minutes at room temperature, and separate the serum from the cells. Please

note that blood sample processing parameters, including time and temperature of blood clotting, centrifugation, and sample storage, may impact measured ECP concentration.[7] Variations in blood processing parameters between sampling may cause inconsistent ECP measurements.

Plasma or hemolyzed samples should not be used.

The use of an ultracentrifuge is recommended to clear lipemic samples.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants.

Volume Required: 5 µL serum

Storage: 7 days at 2–8°C or 3 months at –20°C.[12]

Store the serum samples in polystyrene or polypropylene rather than glass tubes, to avoid a decrease in ECP values. Do *not* attempt to thaw frozen specimens by heating them in a waterbath. Avoid repeated freeze/thaw cycles.

Dilution of High Samples: Samples expected to have ECP levels above the assay's calibration range should be *manually* diluted with ECP Sample Diluent (LEOZ) before assay.

Warnings and Precautions

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Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. Labels on the inside box are needed for the assay.

ECP Bead Pack (L2EO12)
With barcode. 200 beads, coated with monoclonal murine anti-ECP antibody. Stable at 2–8°C until expiration date.
L2KEO2: 1 pack.

ECP Reagent Wedge (L2EOA2)
With barcode. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal rabbit anti-ECP antibody in buffer, with preservative. Stable at 2–8°C until expiration date.
L2KEO2: 1 wedge.

Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

ECP Adjustors (LEOL, LEOH)
Two vials (Low and High) containing lyophilized ECP in an ECP-free nonhuman serum/buffer matrix, with preservative. Reconstitute each vial with **2.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. Aliquot and freeze immediately after reconstitution. Stable at –20°C for 6 months after reconstitution.
L2KEO2: 1 set.

Before making an adjustment, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

ECP Controls (LEOC1, LEOC2)
Two vials containing lyophilized ECP in an ECP-free nonhuman serum/buffer matrix, with preservative. Reconstitute each vial with **2.0 mL** distilled or deionized water. Mix by *gentle, intermittent* swirling. Aliquot and freeze immediately after reconstitution. Stable at –20°C for 6 months after reconstitution. Refer to the

control insert for concentration levels.
L2KEO2: 1 set.

Kit Components Supplied Separately

ECP Sample Diluent (LEOZ)
For the *manual* dilution of patient samples. ECP-free nonhuman serum/buffer matrix, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
LEOZ: 25 mL

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
LRXT: Reaction Tubes (disposable)

Also Required
Distilled or deionized water; test tubes.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual for; preparation, setup, dilutions, adjustment, assay and quality control procedures.

Recommended Adjustment Interval: 2 weeks

Quality Control Samples: Use the control(s) supplied with the kit.

Expected Values

Each laboratory should establish its own reference ranges.

Limitations

Variations in blood processing parameters could cause inconsistent ECP measurements.[7]

Plasma or hemolyzed samples should not be used.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.]

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in ng/mL. (Unless otherwise specified, all results were generated on serum samples collected in Becton Dickinson SST vacutainer tubes.)

Calibration Range: up to 200 ng/mL.

Analytical Sensitivity: 0.2 ng/mL.

High-dose Hook Effect:
None up to 1,591 ng/mL

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three ECP solutions (218, 546 and 983 ng/mL) were assayed. (See "Recovery" table for representative data.)

References

1) D'Amato G, Liccardi G, et al. Measurement of serum levels of eosinophil cationic protein to monitor patients with seasonal respiratory allergy induced by *Parietaria* pollen (treated and untreated with specific immunotherapy). Allergy 1996;51:245-50. 2) Czech W, Krutmann J, et al. Serum eosinophil cationic protein (ECP) is a sensitive measure for disease activity in atopic dermatitis. Br J Dermatol 1992;126:351-5. 3) Juhlin L, Venge P. Eosinophilic Cationic Protein (ECP) in skin disorders. Acta Derm Venereol 1991;71:495-501. 4) Peterson CG, Enander I, et al. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover *in vivo*. Clin Exper Allerg 1991;21:561-7. 5) Reimert CM, Poulsen LK, et al. Measurement of eosinophil cationic protein (ECP) and eosinophil protein X/eosinophil-derived neurotoxin (EPX/EDN). Time and temperature dependent spontaneous release *in vitro* demands standardized sample processing. J Immun Meth 1993;166:183-90. 6) Roquet A, Hallden G, et al. Eosinophil activity markers in peripheral blood have high predictive value for bronchial hyperreactivity in patients with suspected mild asthma. Allergy 1996;51:482-8. 7) Rubira N, Rodrigo MJ, et al. Blood sample processing affect on cationic protein concentraion. Ann Allergy Asthma Immunol 1997;78:394-8. 8) Sunohara N, Furukawa S, et al. Neurotoxicity of human eosinophils towards periperal nerves. J Neurolog Sci 1989;92:1-7. 9) Tomassini M, Magrini L, et al. Serum levels of eosinophil cationic protein in allergic diseases and natural allergen exposure. J Allerg Clin Immun 1996;97:1350-5. 10) Vatrella A, Ponticiello A, et al. Serum eosinophil cationic protein (ECP) as a marker of disease activity and treatment efficacy in seasonal asthma.

Allergy 1996;51:547-55. 11) Wardlaw AJ. Eosinophils in the 1990s: new perspectives on their role in health and disease. Postgrad Med J 1994;70:536-552. 12) Data on file. 13) National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. NCCLS Document H3-A4, Wayne, PA: NCCLS, 1998.

Technical Assistance

In the United States, **available for research use only.** Contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101.

The Quality System of Diagnostic Products Corporation is registered to ISO 13485:2003.

Tables and Graphs

Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	32 in 32	39.9	—	—
	16 in 32	20.7	19.9	104%
	8 in 32	9.55	9.97	96%
	4 in 32	4.86	4.98	98%
	2 in 32	2.36	2.49	95%
	1 in 32	1.20	1.25	96%
2	32 in 32	87.8	—	—
	16 in 32	42.5	43.9	97%
	8 in 32	20.7	21.9	95%
	4 in 32	9.76	11.0	89%
	2 in 32	4.97	5.49	91%
	1 in 32	2.15	2.74	78%
3	16 in 16	60.1	—	—
	8 in 16	28.1	30.0	94%
	4 in 16	13.6	15.0	91%
	2 in 16	6.54	7.51	87%
	1 in 16	2.92	3.76	78%
4	128 in 128	104.8	—	—
	64 in 128	54.2	52.4	103%
	32 in 128	26.4	26.2	101%
	16 in 128	12.9	13.1	98%
	8 in 128	6.32	6.55	96%
	4 in 128	3.23	3.28	98%
	2 in 128	1.51	1.64	92%
	1 in 128	0.7	0.82	85%
5	64 in 64	178	—	—
	32 in 64	83.7	88.9	94%
	16 in 64	41.4	44.4	93%
	8 in 64	20.3	22.2	91%
	4 in 64	10.5	11.1	95%
	2 in 64	4.66	5.55	84%
	1 in 64	2.38	2.78	86%

Recovery (ng/mL)

	Solution	Observed	Expected	%O/E
1	—	27.5	—	—
	A	30.5	38.4	79%
	B	47.5	54.8	87%
	C	78.1	76.7	102%
2	—	30.0	—	—
	A	39.7	40.9	97%
	B	51.0	57.3	89%
	C	73.1	79.1	92%
3	—	43.3	—	—
	A	52.7	54.2	97%
	B	65.7	70.6	93%
	C	88.0	92.5	95%
4	—	48.7	—	—
	A	57.2	59.6	96%
	B	80.1	76.0	105%
	C	107	97.9	109%
5	—	56.9	—	—
	A	62.6	67.8	92%
	B	79.7	84.2	95%
	C	97.0	106	92%
6	—	102	—	—
	A	111	113	98%
	B	134	129	104%
	C	169	151	112%

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