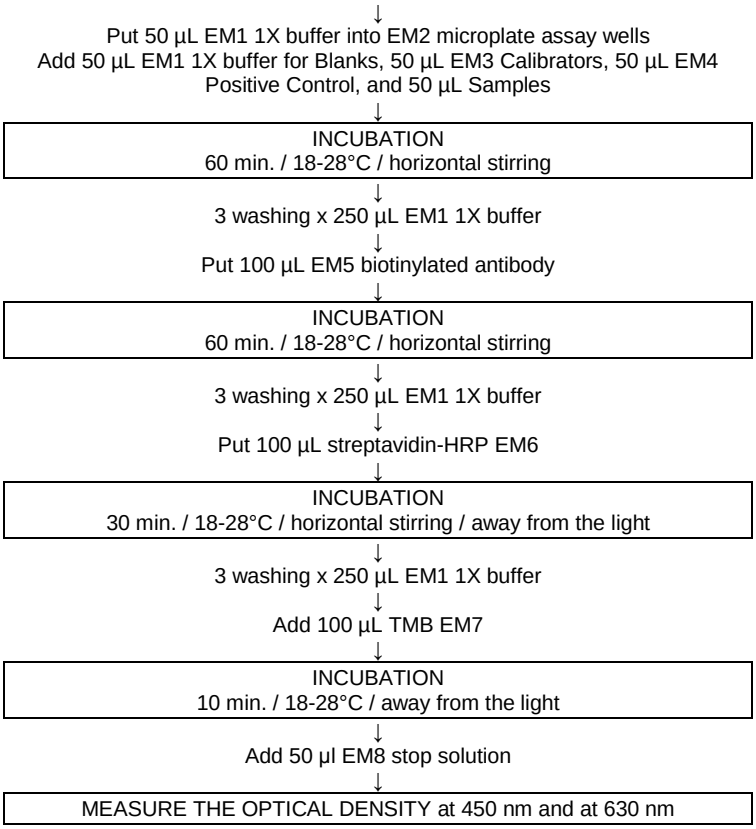
EndoMark® H1 Instructions For Use

Quantitative immuno-enzymatic test for human Endocan / ESM-1 in plasma

Intended use

EndoMark® H1 is an immuno-enzymatic test for a quantitative detection of Endocan / ESM-1 in human plasma. Levels of plasma endocan permit to predict respiratory failure occurrence in septic patients or in polytraumatized patients.

LUNGINNOV SAS
Institut Pasteur de Lille Campus, rue du Professeur Calmette, 59000, Lille, France
Tel : +33 320 877 211 ; Fax : +33 320 877 884
www.lunginnov.com; lunginnov@lunginnov.com



Assay performance summary

Quantification limits	0.3 ng/mL
Linearity	0.3 ng/mL to 5 ng/mL
Analytic sensitivity	0.511 ± 0.084 (DO / $C_{ng/mL}$)
Diagnosis sensitivity	84% (respiratory failure occurrence)
Analytic specificity	90% (endocan in human plasma)
Diagnosis specificity	100% (respiratory failure occurrence)
Repeatability (%CV)	4.80%
Reproducibility (%CV)	7.59%
Interferences	No interference found with a hemolyzed, lactescent or icteric plasma
Detection limits	0.15 ng/mL

Summary and explanation

Endocan, previously called Endothelial cell Specific Molecule 1 (ESM-1), has been identified for the first time in Lille in 1996 (Lassalle et al, 1996). This is a proteoglycan made up of a protein of 165 amino acids (around 20 kDa) and a chondroitin / dermatan sulfate glycanic chain of 15 to 30 kDa covalently bound to the protein on the 137 serine (Bechard et al, 2001a, Delehedde et al, 2006, Sarrazin et al, 2010a). Endocan is specifically secreted by vascular endothelium and preferentially by pulmonary endothelium (Lassalle et al, 1996; Tsai et al, 2002; Bechard et al 2000). The selective expression in pulmonary endothelial cells is controlled by its promoter (Tsai et al, 2002). Lung endothelial cells synthesize and secrete endocan spontaneously. This expression can be increased by proinflammatory cytokines TNFα or IL-1β (Lassalle et al, 1996, Bechard et al, 2000), by bacterial lipopolysaccharide (Scherpereel et al, 2006), or by angiogenic factors like FGF-2 or VEGF (Aitkenhead et al, 2002 ; Scherpereel et al, 2003 ; Grigoriu et al, 2006). By contrast, this expression can be decreased by IFNγ (Lassalle et al, 1996; Bechard et al, 2000) or by angiopoietin 1 via the transcription factor FOXO1 (Dali et al, 2004 et 2006).

The secreted endocan links to the leukocyte integrin LFA-1 and inhibits its interaction with the adhesion molecule ICAM-1 (Bechard et al, 2001b). In the experimental rat endotoxinic shock, the rise of blood endocan induced by treatment with leupeptin (a serine protease inhibitor) is associated to a concomittent decrease of the shock severity, and the rolling and the firm adhesion of leukocytes to the mesenteric vessels (Tissier et al, 2004).

Endocan is an interesting marker to evaluate endothelial dysfunctions in sepsis (Filep et al, 2006; Pierrakos et al, 2010; Paulus et al, 2011). The 4 stages of sepsis are classified by increasing order of severity: SIRS (Systemic Inflammatory Response Syndrome), sepsis, severe sepsis and septic shock (ACCP/SCCM Consensus Conference Committee, 1992). Patients suffering from sepsis, severe sepsis or septic shock have circulating endocan rates superior to 3 ng/mL, whereas in SIRS patients, endocan rates are inferior to 3 ng/mL, with a specificity of 100% (Scherpereel et al, 2006; Paulus et al, 2011).

Endocan is a predictive marker of respiratory failure in septic pathologies and polytraumatized patients. An American prospective study including 48 patients, among which half had presented Acute Respiratory Distress Syndrome (ARDS) after ICU admission. This study pointed out that a rate of endocan < 5 ng predicts the occurrence of ARDS within 3 days after the patient admission in intensive care, whereas a rate of endocan > 7 ng/mL is associated to an absence of ARDS (Mikkelsen et al 2012).

Another prospective study found a similar predictive value of endocan by observing 19 patients suffering from severe sepsis or from septic shock. This study shown that a rate of endocan > 7 ng/mL predicted the occurrence of ARDS within the 48 hours of management, with 84% of sensibility and 100% of specificity (Patent Application WO/2012/098219).

Principle of the procedure

EndoMark® H1 assay is a 4 steps assay that uses (1) a pre-coated microplate with a specific mouse monoclonal antibody against the C-terminal part of human endocan, (2) a monoclonal mouse antibody directed against the N-terminal area of endocan conjugated to biotin, (3) a lectin conjugated to horseradish peroxidase (HRP), (4) a chromogenic substrate.

During the first step, Calibrators, Positive Control and Samples are added into microassay wells, already covered with anti-human endocan monoclonal antibody. The monoclonal antibody binds endocan present in Calibrators, Positive Control or Samples. After the incubation, washing discards unbound materials.

During the second step, the anti-endocan monoclonal antibody conjugated to biotin is added into the wells. The conjugated anti-endocan antibody binds on captured endocan during the first step. After incubation, washing discards unbound conjugated antibody.

During the third step, the lectin conjugated to HRP is added into all the wells. The conjugated lectin binds onto the biotin-conjugated monoclonal antibody immobilized during the second step. After incubation, a washing discards the unbound conjugated lectin.

During the fourth step, the tetramethylbenzidine 3, 3', 5, 5' substrate (TMB) is added into all the wells. The immobilized HRP reacts to the substrate and generate a blue color. After incubation, the colorimetric reaction is chemically stopped; blue color turns yellow, confirming the reaction has been done.

The intensity of the color is measured by a spectrophotometer adjusted on 450 nm (A450). The intensity of the color is proportional to the concentration of endocan in diluted samples, calibrators and positive controls. The results are calculated from the calibrator curve, drawn using the linear regression method.

Reagents and materials provided

The immunoassay kit EndoMark® H1 contains all the following elements:

Component	Nature	Quantity	Reference	Content
EM1	Concentrated buffer solution 20X	75 mL	LIDM-1201-EM1	Contains phosphate buffer (PBS) 20X, 100 mM EDTA, 2% Tween-20® and 0.035% Proclin® 300
EM2	96 wells in divisible strip wells Ready to use	1 bag	LIDM-1201-EM2	6 x 16 wells adsorbed by a monoclonal mouse antibody in an aluminum bag
EM3	Calibrators for human endocan Ready to use 6 Calibrators A : 0.625 ng/mL 6 Calibrators B : 1.25 ng/mL 6 Calibrators C : 2.5 ng/mL 6 Calibrators D : 3.5 ng/mL 6 Calibrators E : 5 ng/mL	0.15 mL in each vial	LIDM-1201-EM3	Contains a known concentration of endocan (ng/ml) in a EM1 1X solution containing 1% of normal human plasma and 0.035% of Proclin® 300
EM4	6 Positive Controls Ready to use	0.15 mL in each vial	LIDM-1201-EM4	Contains a known concentration of endocan (ng/ml) in a EM1 1X solution containing 50% of normal human plasma and 0.035% of Proclin® 300
EM5	Antibody conjugated to biotin Ready to use	12 mL	LIDM-1201-EM5	Contains a conjugate of biotin and anti-endocan mouse monoclonal antibody
EM6	HRP conjugated to streptavidin Ready to use	12 mL	LIDM-1201-EM6	Contains a conjugate of horseradish peroxidase and streptavidin
EM7	TMB substrate Ready to use	12 mL	LIDM-1201-EM7	Contains 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2)
EM8	Stop solution Ready to use	6 mL	LIDM-1201-EM8	Contains sulfuric acid 2N
Adhesive plastic films		2		
Instruction For Use		1		
Certificate of conformity		1		

Tween-20® is a registered trademark of ICI Americas Inc. ProClin® is a registered trademark of Rohm and Haas Company.

Storage and stability of the reagents

Component	State	Storage	Stability
EM1 : concentrated washing buffer	Open	2…8°C	3 months
EM2 : microplate	Open	2…8°C, in the bag with desiccating	2 months
EM3 : calibrators		Storage at -20°C at reception*	12 months at -20°C
EM4 : positive control		Storage at -20°C at reception*	12 months at -20°C
EM5 : biotinylated antibody	Open	2…8°C	3 months
EM6 : streptavidin-HRP	Open	2…8°C	3 months
EM7 : TMB	Open	2…8°C	3 months
EM8 : stop solution	Open	2…8°C	3 months

* Use EM3 and EM4 immediately after thawing. EM3 and EM4 must be eliminated after usage.

Required but not provided materials

- Ultrapure or bidistilled water
- Calibrated container to dilute washing solution
- Washing bottle or another washing material
- Multichannel pipets (8 or 12 channels) or repeat pipettors (optional)
- Micropipettes and tips
- Horizontal orbital stirrer 450 rotations by minute (rbm)
- Timer (60 minutes)
- Plate reader with filters for 450 nm and 630 nm
- Calculator or software to validate and analyze the assay

Precautions

1. For *in vitro* use only.
2. Treat samples as potentially dangerous materials. Samples have to be eliminated in compliance with national procedures on bio risks. Chemical materials must be handled with caution. Treat chemical products as potentially biohazardous material.
3. Wear gloves and protection glasses; follow laboratory procedures when handling the assay.
4. Use all the reagents before the expiratory date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use the microplate if the package is damaged.
7. ProClin® 300 is a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin® 300 can cause irritation to the skin, eyes or mouth. Call a physician if these symptoms occur.
8. The Stop Solution is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
9. Calibrators contains heat-inactivated human plasma coming from Etablissement Français du Sang which are HIV1, HIV2, HCV and HBsAg negative according to the methods approved by the FDA.
10. Positive control contains heat-inactivated human plasma coming from Etablissement Français du Sang which are HIV1, HIV2, HCV and HBsAg negative according to the method approved by the FDA. No method can guarantee the absence of infectious agents; this assay must be handled according to the stage 2 of biologic security as recommended by the book of Centers for Disease Control/National Institutes of Health called "Biosafety in Microbiological and Biomedical Laboratories", 2007 for potentially infectious human plasma or serum.
11. The use of multichannel pipets or repeat pipettors is recommended to ensure the timely delivery of reagents.
12. To measure samples precisely, pipet Samples and Calibrators using calibrated equipment.
13. In order to get precise results, it is required to collect and store properly the Samples (see «*REAGENTS COLLECTION AND PREPARATION*»).
14. Avoid microbial cross contamination of reagents or Samples.
15. Test each Sample in duplicate.
16. Do not use a microassay well for more than one test.
17. Using incubation times and temperatures other than those indicated in the assay procedure may give erroneous results.
18. Keep away TMB substrate from the light during the storage and incubation. Avoid contact with skin, eyes or clothes. If contact is made, wash with water.
19. Do not allow microassay wells to dry while the assay.
20. When removing liquid from the microassay wells do not scrape or touch the bottom of the wells.
21. Use a washing bottle or automatic filling systems to wash microplates (see ASSAY, step 7). Do not use multichannel pipette to wash the microplate.

Storage

WARNING : the kit components must be stored in 2 distinct areas : the box "EM3 / EM4 Calibrators" must be kept at -20°C at reception, the other components must be stored at 2-8°C. After opening, each component has its own expiry date (see « *STORAGE AND REAGENTS STABILITY* ». After use, keep the components at the recommended areas (see « *STORAGE AND REAGENTS STABILITY* »).

Instability or deterioration of reagents indication

Eliminate washing solution if it becomes dirty.

Reagents collection and preparation

Handle and suppress samples following the applicable regulations. Every Sample manipulation can be done at room temperature (18-28°C). Plasma Samples on EDTA are aseptically sampled and then centrifuged following the usual methods[1,2]. The Sample must be essayed immediately or stored at 4°C (24 hours maximum) until the assay. If the Sample cannot be tested within 24 hours, froze it at -20°C. At this storage temperature, plasma endocan from severe septic or septic shock patients remain stable for at least 18 months. More technical information is available on request.

Samples freezing and thawing

Thaw the Samples by putting them at room temperature during 1 hour, and repeat shaking the tubes to homogenize. Thawing can be faster at 37°C. Lunginnov suggests centrifuging tubes at 10-12000 g during 30 sec in order to split non solubilized precipitate and liquid phase of the sample. Put the thawed samples at room temperature before processing the assay. Do not freeze and thaw more than three times. If you need to freeze again the sample for a future analysis, we recommend preparing some aliquots to avoid freezing and thawing cycles.

Samples dilution

Warning: Handle all Samples as potentially infectious. Do not use heat-inactivated, contaminated or poorly preserved samples.

Note: See REAGENTS COLLECTION AND PREPARATION to learn how to freeze properly Samples. A good manipulation is essential to get the most reliable results.

Determine the number (N) of Samples to test. Number the tubes from #1 to #N, and mention the corresponding wells and tubes. Lunginnov suggests to dilute Samples to 1:2 with EM1 1X buffer. The dilution is made directly into the wells by dropping 50 µL pure plasma in the wells that already contained 50 µL EM1 1X buffer. The mixing is made by horizontal orbital stirring of the microplate during the first incubation. Samples with absorbance values [A450 – A630] superior to the highest absorbance value of calibrator [A450 – A630] has to be tested again with a dilution at 1:4 or 1:8.

Sample addition into wells

This manipulation has to be realized in less than 20 minutes. 50 µL Samples, 50 µL Calibrators, 50 µL Positive Control and 50 µL EM1 1X buffer are directly added into the wells that already contain 50 µL EM1 1X buffer with a pipet (see step 2 to 5 of the ASSAY procedure). If there are a lot of Samples, it is recommended to use a multichannel pipet.

A 25 mL reservoir method can be used to add EM1 1X buffer, EM5 conjugated antibody, EMO conjugated lectin, EM7 substrate and EM8 stop solution with a multichannel pipet.

Reagent preparation

Equilibrate all the reagents to 18–28°C prior to use. After use, close packaging and store the different components and unused reagents to required temperature (see *STORAGE*).

Positive Control and Calibrators

Calibrators and Positive Control contain a known concentration of recombinant endocan controlled by internal certified calibrator. Calibrators and positive control do not need dilution or preparation before use. Concentration is indicated on tubes and on the certificate of conformity.

Washing solution

Mix 20X concentrated EM1 buffer by reversing several times the bottle. When EM1 20X buffer is stored at 2-8°C, some precipitates can appear. In order to dissolve the precipitates, heat the bottle in a water bath at 37-50°C until dissolution. Prepare EM1 1X buffer by diluting EM1 20X in ultrapure or bidistilled water, so as to obtain a final volume adapted to the number of Samples that have to be tested. Mix well. The washing solution is stable for 30 days at 2-8°C. Suppress the reagent in case of cloudiness.

Stripwells

Determine the number of strips needed for the assay. It is recommended to test Blank, Positive Control, Calibrators and Samples in duplicate. Close the package and store at 2-8°C. Put the strips for the assay on the stand.

Assay

Read the entire procedure before beginning the assay.

[1] Center for Disease Control. « Recommendations for prevention of HIV transmission in health-care settings », MMWR. 1987 36(suppl. n° 2S):001

[2] WHO/DIL/LAB/99.1 Rev.2

See REAGENTS PREPARATION *and PRECAUTIONS.*
1. Write down the localization of the wells that correspond to Blank, Calibrators, Positive Control, and Samples, and the reference number indicated on tubes. Add 50 µL EM1 1X buffer into all the wells.
2. Add 50 µL EM1 1X buffer into Blank wells.
3. Add 50 µL each EM3 endocan Calibrators in duplicate (calibrators A, B, C, D and E). NOTE: Calibrators are ready to use, no dilution is needed.
4. Add 50 µL EM4 Positive Control into the corresponding wells. Note: Positive Control is ready to use and does not need to be diluted.
5. Add 50 µL each Samples into the corresponding wells. (See *SAMPLES DILUTION*).
6. Incubate at 18-28°C 60 ± 1 minute, on horizontal stirring at 450 rotations per minute.
7. Wash the wells following these steps:
    a. After step 6 (or step 10 and 13 here below), remove all the liquid from the wells.
    b. Add around 250 µL EM1 1X buffer in each well, with a washing bottle or an automatic system.
    c. Remove all the liquid from the wells.
    d. Repeat b-c steps twice.
    e. After the third washing, reverse the wells and tap firmly on absorbing paper to eliminate all residual liquid.
8. With a multichannel pipet or repeat pipettors, put into each well 100 µL EM5 conjugated anti-endocan antibody including Blank wells.
9. Incubate the stripwells at 18-28°C during 60 ± 1 minute on horizontal stirring at 450 rotations per minute.
10. After incubation, repeat the washing step 7.
11. With a multichannel pipet or repeat pipettors, put into each well 100 µL EM6 conjugated lectin including Blank wells.
12. Incubate the stripwells at 18-28°C during 30 ± 1 minute on horizontal stirring at 450 rotations per minute away from the light.
13. After incubation, repeat the washing step 7.
14. Immediately after washing, put 100 µL EM7 Substrate Solution in all the wells.
15. Incubate the stripwells at 18-28°C during 10 ± 1 minute away from the light.
16. Add 50 µL EM8 Stop Solution in each well to stop the enzymatic reaction. Add EM8 Stop Solution at the same speed and order than the substrate solution. Tap the plate slowly to allow a homogeneous coloration.
17. Read the absorbance at 450 nm (value A450) then at 630 nm (value A630) for each well within 30 minutes following addition of Stop Solution (step 16), and make a subtraction [A450 – A630].
18. Read Positive Control and Sample concentration with the calibration curve.
19. Eliminate the used stripwells and the residual diluted samples (see PRECAUTION).

Quality control

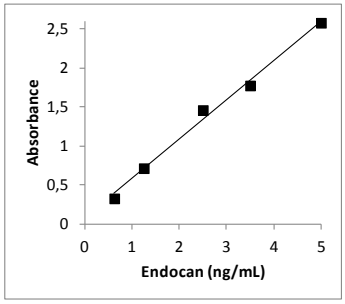
Laboratory methods recommend making controls to make sure the assay works. Each EndoMark® H1 kit contains a Positive Control for this purpose. For this control, the value range is provided (see EM4 certificate of conformity). Positive control value validates the calibration curve and the samples results. Each laboratory has to establish its own validation standards. If the obtained value exceeds the limits, the results will be considered as unlikely, and another assay will have to be done. The Instructions For Use included into the kit provides several acceptation standards. If the assay does not respect it, do it again or contact Lunginnov technical service. The Certificate of Conformity included into the kit refers to a lot number and has to be used to check if the results obtained correspond to the Lunginnov's results. Optical densities are mentioned only as a guide. Laboratory results can be different.

Results interpretation

Results calculation

Calibration curve calculation

The regression line equation (y = ax + b) is calculated by putting on the y axis absorbance values [A450 – A630] of each Calibrator (Blank value need to be subtracted first), and on the x axis the corresponding concentration. Calibration curve has to be in compliance with validation standards (see VALIDATION). Most of computers can make these calculations. Here below is an example of calibration curve.



Calibrator E: 5 ng/mL, absorbance = 2.580
Calibrator D: 3.5 ng/mL, absorbance = 1.775
Calibrator C: 2.5 ng/mL, absorbance = 1.460
Calibrator B: 1.25 ng/mL, absorbance = 0.714
Calibrator A: 0.625 ng/mL, absorbance = 0.326
White 0 ng/mL, absorbance = 0.055
r = 0.996 ; a = 0.505 ; b = 0.0718

Regression line equation: y =0.505x + 0.0718 (r^2 = 0.992) where y corresponds to absorbance value [A450 – A630], and x to the concentration found from the observed absorbance value A450.

Calculation of endocan concentration in samples

To calculate endocan concentration in a sample, multiply the concentration (obtained with the calibration curve) by the dilution factor 2. For example, if for one plasma diluted at 1:2, an endocan concentration of 2.5 ng/ml is obtained, the final endocan concentration in the sample will be 5 ng/ml (or 2 x 2.5).

If the value [A450 – A630] of a sample is superior to the one of the highest Calibrator 1, the sample need to be diluted and tested again to get values [A450 – A630] inferior to Calibrator 1. Calibrators and Controls will have to be tested again too.

Validation

Determine the slope, interception and correlation coefficient of the line obtained using Calibrators A, B, C, D and E. To validate the assay, values have to be within this range:

- Correlation coefficient (r): > 0.97
- Slope (a): between 0.411 and 0.764
- Interception with y axis (b): between -0.381 and 0.439
- Blank < 0.100

See tube labels to find the endocan concentrations.

Limitations

EndoMark® H1 assay has been used on plasma sample collected on EDTA. No other anticoagulant has been tested.

Clinical interpretation

In a SIRS patient, a plasma endocan > 3 ng/mL is suspect of sepsis with a 100% specificity (n=63, Scherpereel et al, 2006; Paulus et al, 2011).

in patient admitted in intensive care unit for severe sepsis, septic shock or severe polytrauma, the rate of circulating endocan predicts the occurrence of respiratory failure at 48h to 72h:

- An endocan rate > 7 ng/mL is associated to a low risk of occurrence of respiratory failure in polytraumatized patients (n=48, specificity 100%, Mikkelsen et al, 2012) ;
- An endocan rate < 5 ng/mL is associated to a high risk of occurrence of respiratory failure at 48h to 72h :
    - In polytrauma patient (n=48 ; odd ratio = 0.69 ; confidence interval 0.49-0.97; p = .03, Mikkelsen et al, 2012)
    - In severe sepsis patient (n=19, sensibility 84%, specificity 100%, Patent Application WO/2012/098219).

Values found

An assay of plasma and serum endocan including 32 healthy donors has been made. Here below are the results. Values are given in ng/mL.

	N	Average	Standard deviation	Median	3rd quartile	Extreme values
Plasma	32	1,00	0,33	1,00	1.33	0.40 – 1.80
Serum	32	1,18	0,46	1,15	1.43	0.40 – 2.90

Note: Endocan concentration for plasma and serum samples can change according to the laboratories. It is better that each laboratory define its own standard values. Concentrations above are only mentioned as a guide.

Assay characteristics

Limits

LD: Detection limit (LD) of endocan assay is 0.15 ng/ml. The calculation is made following standardization data and ICH[3] guide recommendation.
LQ: Quantification limit (LQ) of endocan assay is 0.3 ng/ml. This is the smallest concentration read on the calibrator curve, calculated following standardization data and ICH guide recommendation.

Interfering materials

Overload tests – dilution had been made on icteric, hemolyzed or lactescent plasmas and serums. There is no interference of these samples on endocan assay.

	n*	Linearity	Average covering (%)	Min-Max
Icterus	3	3/3 Linear 1:2 – 1:16	106%	95%-114%
Hemolysis	8	8/8 Linear 1:2 – 1:16	101%	87%-113%
Lactescence	8	8/8 Linear 1:2 – 1:16	94%	83%-105%
Clear	6	6/6 Linear 1:2 – 1:16	107%	98%-115%
Undetermined	2	2/2 Linear 1:2 – 1:16	94%	93%-96%

*** : 3 samples were both lactescent and hemolyzed.**

Fidelity

The intra-essay repeatability was performed on 10 plasmas from severe sepsis or septic shock patients in 6 replicates. Results show an %CV average intra-assay at 4.80%.

Patient n°	replicate 1	replicate 2	replicate 3	replicate 4	replicate 5	replicate 6	Ave.	ET	%CV
P1	4.900	5.010	4.950	5.040	4.930	4.910	4.957	0.056	1%
P2	1.290	1.300	1.310	1.240	1.280	1.280	1.283	0.024	2%
P3	4.170	4.210	4.330	4.490	4.300	4.320	4.303	0.112	3%

[3] ICH, International Conférence on Harmonisation ; Validation of analytical procedures : text and methodology (Q2(R1), revision 2005.

P4	1.560	1.650	1.670	1.660	1.610	1.730	1.647	0.058	3%
P5	2.660	2.720	2.710	2.710	2.600	2.690	2.682	0.045	2%
P6	1.280	1.310	1.300	1.410	1.310	1.390	1.333	0.053	4%
P7	0.982	0.971	0.971	0.961	0.947	1.010	0.974	0.021	2%
P8	1.540	1.640	1.660	1.740	1.720	1.670	1.662	0.071	4%
P9	0.975	1.020	1.010	1.020	1.310	1.340	1.113	0.166	15%
P10	3.260	3.300	3.310	3.410	4.340	3.390	3.502	0.415	12%

Repeatability values of endocan in ng/mL. %CV = 100 x ET/m.

The inter-essay reproducibility study was performed on 10 severe sepsis or septic shock patients' plasma made 5 times at different days and by 2 different operators. Results show a %CV average inter-assay at 7.59%.

Operator 1								
Patient	n=1	n=2	n=3	n=4	n=5	Moy	ET	%CV
P1	4.6	5.52	4.65	4.96	4.96	4.937	0.366	7.42%
P2	1.31	1.38	1.17	1.28	1.05	1.239	0.130	10.48%
P3	4.3	4.23	4.06	4.30	4.16	4.211	0.103	2.44%
P4	1.65	1.82	1.53	1.65	1.29	1.587	0.196	12.33%
P5	2.71	2.92	2.55	2.68	2.49	2.670	0.167	6.24%
P6	1.27	1.4	1.22	1.33	1.06	1.257	0.129	10.27%
P7	0.938	1	0.874	0.97	0.74	0.905	0.104	11.46%
P8	1.76	1.56	1.42	1.66	1.38	1.556	0.160	10.27%
P9	0.964	1.24	0.919	1.11	0.8	1.007	0.172	17.04%
P10	3.15	3.61	3.27	3.50	3.17	3.340	0.206	6.15%
Operator 2								
Patient	n=1	n=2	n=3	n=4	n=5	Moy	ET	%CV
P1	4.55	4.79	4.42	4.74	4.45	4.590	0.168	3.66%
P2	1.11	1.01	1.18	1.19	1.1	1.118	0.073	6.49%
P3	4.13	4.21	3.7	4.16	3.78	3.996	0.237	5.93%
P4	1.42	1.46	1.51	1.61	1.44	1.488	0.076	5.10%
P5	2.28	2.71	2.59	2.37	2.51	2.492	0.171	6.87%
P6	1.1	1.1	1.21	1.11	1.2	1.144	0.056	4.89%
P7	0.76	0.728	0.87	0.9	0.85	0.822	0.074	9.00%
P8	1.34	1.47	1.42	1.43	1.45	1.422	0.050	3.50%
P9	0.85	0.87	1.01	0.932	0.94	0.920	0.063	6.88%
P10	2.7	3.11	3.02	3.03	2.92	2.956	0.158	5.35%
							average	**7.59%**
							ET	**3.54%**
							Min	**2.44%**
							Max	**17.04%**

Reproducibility values of endocan in ng/mL. %CV = 100 x ET/m.

Linearity

The linearity was evaluated by overloading 10 plasma samples with 10 ng/mL recombinant endocan, serial diluting the plasma with EM1 1X buffer, and comparing the values with the expected ones.

		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
	Endocan concentration in ng/mL with dilution factor taken into account										
2	Overload plasma	12.18	11.39	12.06	11.47	11.43	11.32	10.76	11.50	10.91	10.31
4		14.12	10.23	13.59	11.27	11.27	10.23	9.85	11.05	10.15	13.37
8		13.42	9.53	12.15	10.71	10.71	9.99	8.92	10.14	9.08	10.57
16		12.93	9.85	11.46	9.34	9.34	9.13	8.92	9.64	8.65	10.54
2	Not overload plasma	4.33	0.94	3.73	2.27	2.27	1.02	0.60	1.18	0.70	2.95
2		4.55	0.97	3.95	2.41	2.41	1.00	0.72	1.22	0.71	3.16
	Overload test : % covering										
	Overload plasma	13.16	10.25	12.32	10.70	10.69	10.17	9.61	10.58	9.70	11.20
	Not overload plasma	4.44	0.96	3.84	2.34	2.34	1.01	0.66	1.20	0.70	3.06
	% Covering	91%	94%	89%	87%	87%	92%	90%	94%	91%	86%
	Dilution test : % of covering for each dilution calculation										
2	Overload plasma	84%	104%	87%	93%	93%	103%	101%	103%	102%	79%
4		98%	93%	98%	91%	91%	93%	92%	99%	95%	102%
8		93%	87%	88%	87%	87%	91%	84%	91%	85%	81%
16		90%	90%	83%	76%	76%	83%	84%	86%	81%	81%

Overload test – dilution on septic plasma. Concentrations are in ng/mL. %Rec = 100 x [concentration found]/[concentration expected = 10 + endocan endogen].

Overload test on severe sepsis or septic shock patient plasma show an average %Rec at 90% ± 3.00%. Each value (n=60) of the dilution test on plasma overloaded with endocan demonstrate an acceptable linearity.

Support

For commercial queries please contact commercial@lunginnov.com. For technical support, please contact technical@lunginnov.com. More information on Lunginnov's products and distributors are available on www.lunginnov.com.

Publication date

APRIL, 4TH, 2014

References

ACCP/SCCM Consensus Conference Committee (1992). *Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis*. Chest 101:1644-65

Aitkenhead M, Wang SJ, Nakatsu MN, Mestas J, Heard C and Hughes CC (2002) *Identification of endothelial cell genes expressed in an in vitro model of angiogenesis: induction of ESM-1, (beta)ig-h3, and NrCAM*. Microvasc Res. 63(2):159-171.

Bechard D, Gentina T, Delehedde M, Scherpereel A, Lyon M, Aumercier M, Vazeux R, Richet C, Degand P, Jude B, Janin A, Fernig DG, Tonnel AB and Lassalle P (2001a) *Endocan is a novel chondroitin sulfate/dermatan sulfate proteoglycan that promotes hepatocyte growth factor/scatter factor mitogenic activity*. J. Biol. Chem. 276(51):48341-48349.

Bechard D, Meignin V, Scherpereel A, Oudin S, Kervoaze G, Bertheau P, Janin A, Tonnel AB and Lassalle P (2000) *Characterization of the secreted form of endothelial-cell-specific molecule 1 by specific monoclonal antibodies*. J Vasc Res. 37(5):417-425.

Bechard D, Scherpereel A, Hammad H, Gentina T, Tsicopoulos A, Aumercier M, Pestel J, Dessaint JP, Tonnel AB and Lassalle P (2001b) *Human endothelial-cell specific molecule-1 binds directly to the integrin CD11a/CD18 (LFA-1) and blocks binding to intercellular adhesion molecule-1*. J. Immunol. 167(6):3099-3106.

Daly C, Pasnikowski E, Burova E, Wong V, Aldrich TH, Griffiths J, Ioffe E, Daly TJ, Fandl JP, Papadopoulos N, McDonald DM, Thurston G, Yancopoulos GD and Rudge JS (2006) *Angiopoietin-2 functions as an autocrine protective factor in stressed endothelial cells*. Proc Natl Acad Sci U S A. 103(42):15491-6.

Daly C, Wong V, Burova E, Wei Y, Zabski S, Griffiths J, Lai KM, Lin HC, Ioffe E, Yancopoulos GD and Rudge JS (2004) *Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1)*. Genes Dev. 18(9):1060-1071.

Delehedde M, Sarrazin S, Adam E, Motte V and Vanpouille C (2006) *Proteoglycans and glycosaminoglycans : Complex molecules with modulating activity. In: Delehedde & Lortat-Jacob, eds. New Developments in Therapeutic Glycomics*. Kerala: Research Signpost, pp 1-13.

Depontieu F, Grigoriu BD, Scherpereel A, Adam E, Delehedde M, Gosset P and Lassalle P (2008) *Loss of Endocan tumorigenic properties after alternative splicing of exon 2*. BMC Cancer. 8:14-21

Filep JG (2006) *Endocan or endothelial cell-specific molecule-1: a novel prognostic marker of sepsis ?*. Crit Care Med. 34(2):574-575

Grigoriu BD, Depontieu F, Scherpereel A, Gourcerol D, Devos P, Ouatas T, Lafitte JJ, Copin MC, Tonnel AB and Lassalle P (2006) *Endocan expression and relationship with survival in human non-small cell lung cancer*. Clin. Cancer Res. 12(15):4575-4582.

Janke J, Engeli S, Gorzelniak K, Feldpausch M, Heintze U, Bohnke J, Wellner M, Herse F, Lassalle P, Luft FC and Sharma AM (2006) *Adipose tissue and circulating endothelial cell specific molecule-1 in human obesity*. Horm Metab Res. 38(1):28-33.

Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, Devos R and Tonnel AB (1996) *ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines*. J. Biol. Chem. 271:20458-20464

Mikkelsen ME, Shah C, Scherpereel A, Lanken PN, Lassalle P, Bellamy SL, A. Localio R, Albelda SM, Meyer NJ and Christie JD (2011) *Lower Serum Endocan Levels Are Associated with the Development of Acute Lung Injury after Major Trauma.* J. Crit. Care: In Press, July 2011

Paulus P, Jennewein C, Zacharowski K (2011) Biomarkers of endothelial dysfunction: can they help us deciphering systemic inflammation and sepsis?. Biomarkers. 2011 Jul;16 Suppl 1:S11-21. Review.

Pierrakos C and Vincent JL (2010) *Sepsis biomarkers: a review.* Crit. Care 14(1):R15

Reikvam H, Hatfield KJ, Lassalle P, Kittang AO, Ersvaer E and Bruserud Ø (2010) *Targeting the angiopoietin (Ang)/Tie-2 pathway in the crosstalk between acute myeloid leukaemia and endothelial cells: studies of Tie-2 blocking antibodies, exogenous Ang-2 and inhibition of constitutive agonistic Ang-1 release*. Expert Opin Investig Drugs. (2):169-183

Reinhart K, Meisner M and Brunkhorst FM (2006) *Markers for sepsis diagnosis: what is useful ?* Crit Care Clin. 22(3):503-519.

Sarrazin S, Adam E, Lyon M, Depontieu F, Motte V, Landolfi C, Lortat-Jacob H, Bechard D, Lassalle P and Delehedde M (2006) *Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy*. Biochim. Biophys. Acta Reviews 1765(1):25-37

Sarrazin S, Lyon M, Deakin JA, Guerrini M, Lassalle P, Delehedde M and Lortat-Jacob H (2010a) *Characterization and binding activity of the chondroitin/dermatan sulfate chain from Endocan, a soluble endothelial proteoglycan.* Glycobiology. 20(11):1380-1388

Scherpereel A, Depontieu F, Grigoriu B, Cavestri B, Tsicopoulos A, Gentina T, Jourdain M, Pugin J, Tonnel AB and Lassalle P (2006) *Endocan, a new endothelial marker in human sepsis*. Crit. Care Med. 34(2):532-537.

Scherpereel A, Gentina T, Grigoriu B, Senechal S, Janin A, Tsicopoulos A, Plenat F, Bechard D, Tonnel AB and Lassalle P (2003) *Overexpression of endocan induces tumor formation*. Cancer Res. 63(18):6084-6089

Shin JW, Huggenberger R and Detmar M (2008) *Transcriptional profiling of VEGF-A and VEGF-C target genes in lymphatic endothelium reveals endothelial-specific molecule-1 as a novel mediator of lymphangiogenesis*. Blood. 112(6):2318-2326

Tissier S, Lancel S, Marechal X, Mordon S, Depontieu F, Scherpereel A, Chopin C and Neviere R (2004) *Calpain inhibitors improve myocardial dysfunction and inflammation induced by endotoxin in rats*. Shock. 21(4):352-357.

Tsai JC, Zhang J, Minami T, Voland C, Zhao S, Yi X, Lassalle P, Oettgen P and Aird WC (2002) *Cloning and characterization of the human lung endothelial-cell-specific molecule-1 promoter***.** J Vasc Res. 39(2):148-159.

Lexic

The used pictograms are in compliance with ISO EN 980 standard and with EDMA guide « User Awareness of Symbols, revision january 2011 »[4].

Used pictograms	Signification*	Used pictograms	Signification*
	In Vitro Diagnostic Medical Device		CE Logo
	Manufacturer		Temperature limitation
	Use until AAAA-MM-JJ		Enough for
	Lot code		Catalog reference
	Caution consult Instructions For Use		

*: Signification in compliance with EDMA guide « *User Awareness of Symbols, revision January 2011* »[5]

[4] http://www.edma-ivd.be/fileadmin/upl_documents/Symbols/2011_Jan_Awareness_of_symbols_Rev_GUI_PUB.pdf