Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a protein cytokine that was discovered in the 1980s. It is also known as B-cell stimulatory factor 2, hepatocytestimulating factor, hybridoma growth factor, or interferon (IFN)-β2. IL-6 participates in inflammation, immune response, and acts in the coordination of developmental, neuronal, and metabolic processes (1). IL-6 acts as a transmitter of alarm signals to the whole organism, indicating the occurrence of an emergency such as infection or tissue damage. Human IL-6 is made up of 212 amino acids, including a 28-amino-acid signal peptide, and its gene has been mapped to chromosome 7p21. Although the core protein is 20 kDa, glycosylation accounts for the size of 21–26 kDa of natural IL-6.

Interestingly, IL-6 can exert pro-inflammatory as well as anti-inflammatory signals, depending on the receptor complex with which it interacts. IL-6 interacts with IL-6 receptor α and this binary complex then further binds to gp 130. The resulting hexameric complex is capable of downstream signaling. The IL-6 receptor α can function as membrane-bound proteins and also exist in soluble form (2). Depending on the form of IL-6 receptor, IL-6 can transmit anti-inflammatory messages (by binding of IL-6 to IL-6 receptor α in the cell membrane) or pro-inflammatory ones (by binding to a soluble form of IL-6 receptor α). Gp130 is a membrane-bound co-receptor that is expressed in various cell types whereas IL-6 receptor exists in membrane-bound form only on certain cell types, such as hepatocytes, neutrophils, T-cells, or monocytes (2).

IL-6 acts at the very beginning of the inflammation process, stimulating upregulation of acute-phase proteins such as C-reactive protein, serum amyloid A, fibrinogen, and haptoglobin in hepatocytes. IL-6 also plays an important role in acquired immune response by the stimulation of antibody production and effector T-cell development. The balance between IL-6 interaction with soluble and membrane-bound forms of IL-6 receptor largely determines pro-inflammatory and anti-inflammatory activities of this cytokine (3).

Clinical value of IL-6

IL-6 has been shown to be involved in many physiological activities, disease initiation and progression, and the pleiotropic nature of this cytokine makes it a key player in many physiologic processes (4). IL-6 is involved in hematopoiesis, and neuronal cells proliferation (5.6). Atherosclerosis is believed to include inflammation processes so that IL-6 has been used as a marker of cardiovascular risk (7). Increased IL-6 levels are strongly correlated with hypertension, dyslipidemia, and glucose resistance (8).

It came as no surprise that serum IL-6 levels were also upregulated during the recent outbreak of the latest Coronavirus infection (SARS-CoV-2). According to Gong et al., IL-6 levels were significantly lower in mild cases compared with severe cases and critically ill groups of patients with SARS-CoV-2 (9). IL-6 levels are associated with the severity of the COVID-19 infection (10, 11). Moreover, IL-6 could be a predictive marker of survival in COVID-19 patients outperforming CRP, D-dimer, and ferritin, independently of demographics and comorbidities (12). The determination of IL-6 levels in human blood is primarily accomplished with the use of sandwich type immunoassays. Baseline levels of human IL-6 in the blood are known to be in single pg per ml digits and can increase up to thousands of pg/ml upon severe sepsis (13). Therefore, assays characterized by high sensitivity and a wide diagnostic window are needed for the reliable determination of IL-6 in the bloodstream.

CLINICAL UTILITY
Systemic inflammation
Sepsis

Reagents for the IL-6 immunoassay development

Hytest provides several monoclonal antibodies that are capable of detecting both recombinant human IL-6 (Cat.# 8IL6) and native IL-6 in serum.

Monoclonal antibodies were developed using full-length human recombinant IL-6 as an immunogen and mice, rats, and rabbits as the source of the immune cells. All of the developed MAbs are capable of working in a sandwich chemiluminescent assay with streptavidin -polyHRP. The recommended MAb pairs are described in Table 1, 2 and 3.

Table 1. ***Recommended MAb pairs to be used in a chemilumines-cent sandwich ELISA with Streptavidin-polyHRP (15 minute assay time).***

Coating antibody	Detector antibody	LoD, pg/ml
L152	L137	0.7
L143	L395	0.4
L519	L395	0.7
L143	L106	0.4
L152	L395	0.5

Table 2. ***Recommended MAb pairs to be used in a chemilumines-cent sandwich ELISA with Acridinium ester or Alkaline Phosphatase.***

Coating antibody	Detector antibody
L152	L137
L152	L106
L143	L106

Table 3. ***Recommended MAb pairs to be used in a lateral flow assay with fluorescent label.***

Coating antibody	Detector antibody
L395	L152
L143	L395

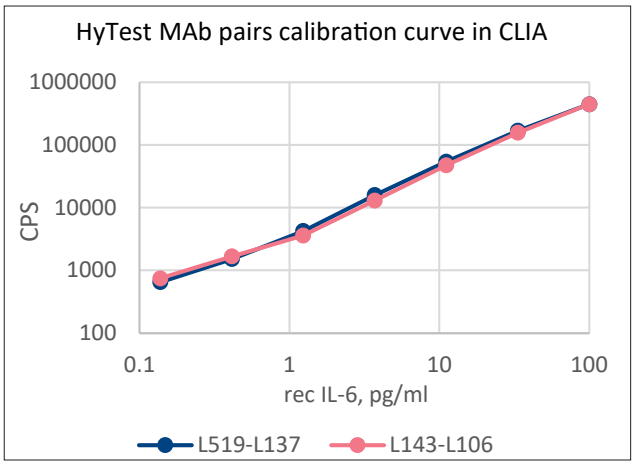

Figure 1.
Calibration curve of Hytest MAb pairs L519-L137 and L143-L106 (capture-detection) with recombinant IL-6. *CLIA with Streptavidin-polyHRP was used. Coating MAbs 200 ng/well, biotinylated MAbs 50 ng/well for L137 and 100 ng/well for L106. The incubation time was 15 minutes (diluent buffer: PBS+7.5% BSA).*

Hytest's MAb pairs demonstrate excellent sensitivity when used for the determination of recIL-6 concentration in 1-100 pg/ml range (see Figure 1).

IL-6 levels can increase up to 10 ng/ml during severe septic conditions (14). Therefore, it is important for a clinician to have an opportunity to detect IL-6 concentrations that are high in the immunoassays without prior dilution. The MAb pairs provided by Hytest can offer a wide linearity range together with high sensitivity (see Figure 2).

When recombinant IL-6 is titrated in a sandwich CLIA along with native IL-6, both titration curves go in parallel. This indicates that the interaction of MAb pairs with recombinant IL-6 is similar to that of native IL-6 (see Figure 3).

To test Hytest MAb pairs in a clinical setting, we used a collection of blood samples of septic patients, both serum and plasma (N=40), and we determined IL-6 levels using several Hytest MAb pairs (see Figure 4). Correlation analysis of data collected with IL-6 levels was determined in the same set of samples with the Roche Cobas 6000 IL-6 assay (correlation coefficient R^2=0.9954). Hytest's MAb pairs could be used for detecting IL-6 levels both in human serum and human plasma, and they demonstrate quantitative results that correlate well with IL-6 data collected using the Roche Cobas 6000 analyzer.

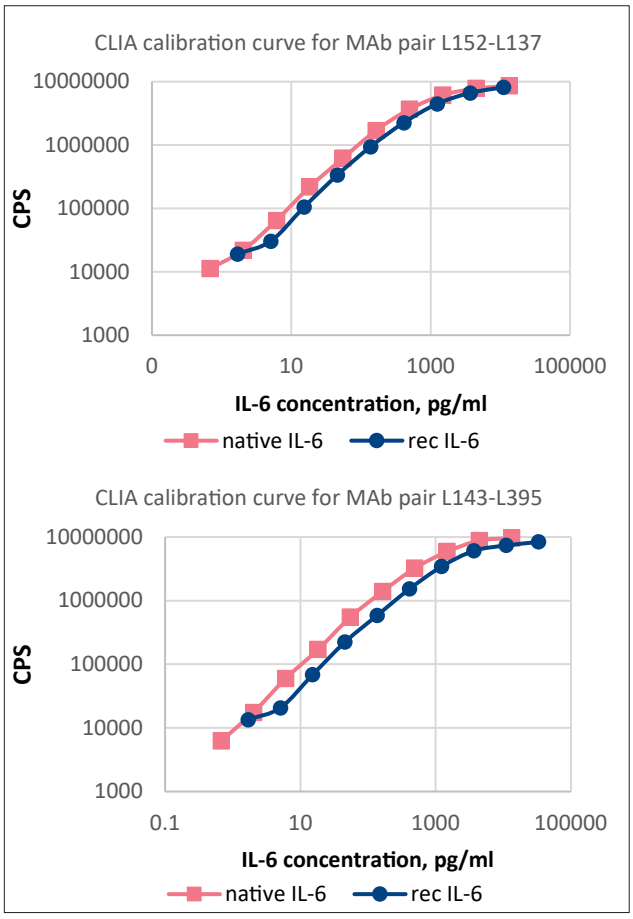

Figure 2.
Calibration curve for MAb pairs L152-L137 and L143-L395. *CLIA with Streptavidin-polyHRP was used. Recombinant IL-6 and native IL-6 were taken as a calibrator. For native IL-6, mononuclear cells were isolated from the blood of healthy human donors, cultivated in culture, and stimulated with bacterial lipopolysaccharide. The concentration of native IL-6 in conditioned media was determined by the Roche Cobas 6000 analyzer. Coating MAbs 200 ng/well, biotinylated MAbs 100 ng/well. The incubation time was 60 minutes (diluent buffer: PBS+7.5% BSA).*

The testing of another set of clinical samples (N=67) in CLIA with Hytest's MAb pairs using acridinium ester as a label demonstrated an even better correlation with the Roche IL-6 assay (see Figure 5).

Moreover, the Hytest MAb pair L152-L137 demon-strated a good correlation in CLIA with the Siemens IMMULITE 2000 IL-6 assay when used for the determination of IL-6 levels in a group of patients (N=107) (see Figure 6).

To check for cross-reactivity, a panel of cognate human proteins was used. IL1α, IL1β, IL2, IL3, IL4, IL8, INFg, TNFα at a concentration of 50 ng/ml were used for cross-reactivity testing in sandwich CLIA. For all of the MAb pairs tested, the cross-reactivity level did not exceed 0.11%.

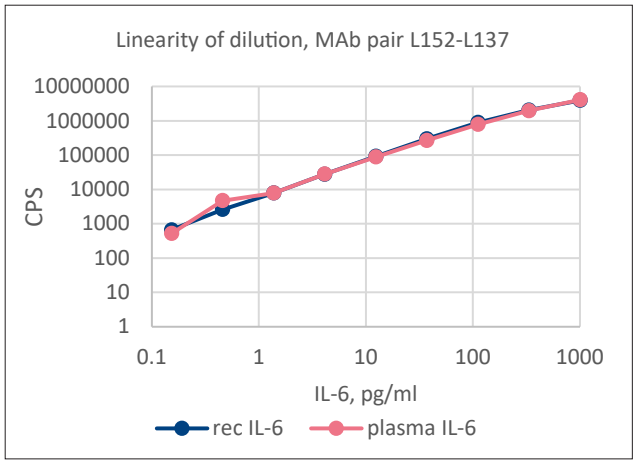

Figure 3.
Dilution linearity of recombinant IL-6 and IL-6 from the plasma of septic patients, measured in sandwich CLIA with MAb pair L152-L137. *CLIA with Streptavidin-polyHRP was used. Plasma dilution was made in parallel for rec IL-6 and plasma IL-6. Plasma IL-6 concentration was measured with the Roche Cobas 6000 analyzer.*

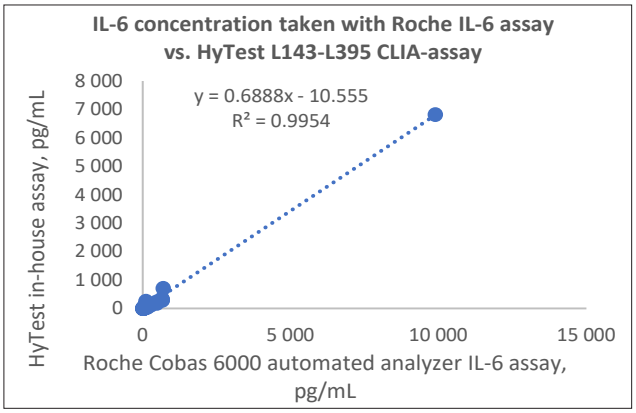

Figure 4.
Testing of the blood samples of septic patients with Hytest's MAb pair L143-L395 and the comparison to IL-6 mass concentration was determined in the same set of samples with the Roche Cobas 6000 IL-6 assay. *CLIA with Streptavidin-polyHRP was used. Undiluted (all of the samples but one) serum or plasma were used in assays. Recombinant IL-6 was used as a calibrator in Hytest's L143-L395 assay. Coating MAbs 200 ng/well, biotinylated MAbs100 ng/well (diluent buffer: PBS+7.5% BSA). The incubation time was 1 hour for the Hytest MAb pair. The Roche Cobas 6000 IL-6 assay was used according to the manufacturer's instructions.*

Hytest's MAbs could be used to construct Lateral Flow assays for the quantitative determination of IL-6 (see Figure 7).

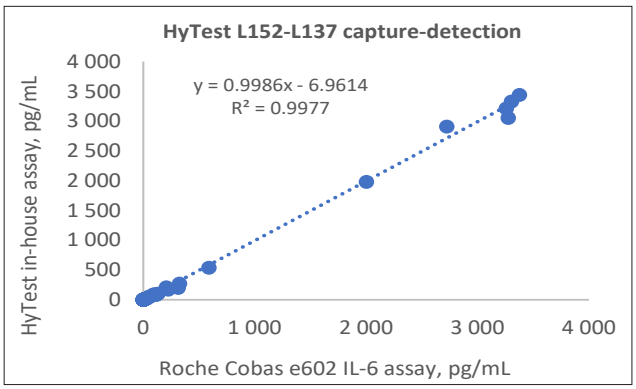

Figure 5.
Determination of IL-6 in the clinical samples of patients with the Hytest MAb pair L152-L137 and correlation to the Roche IL-6 data. *CLIA with acridinium ester as a label was used for the Hytest MAb pair.*

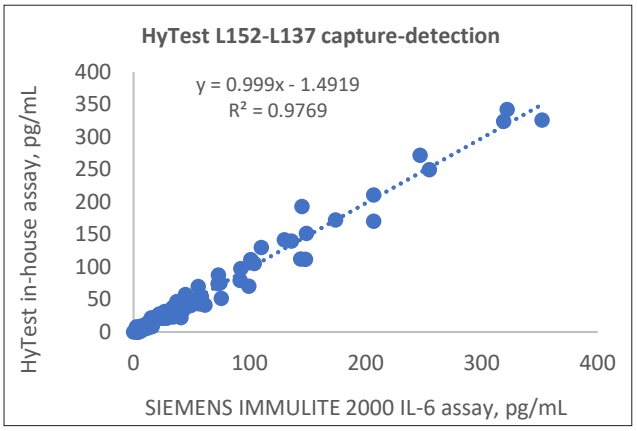

Figure 6.
Determination of IL-6 in the clinical samples of patients with the Hytest MAb pair L152-L137 and correlation to the Siemens IMMULITE 2000 IL-6 data. *CLIA with acridinium ester as a label was used for the Hytest MAb pair.*

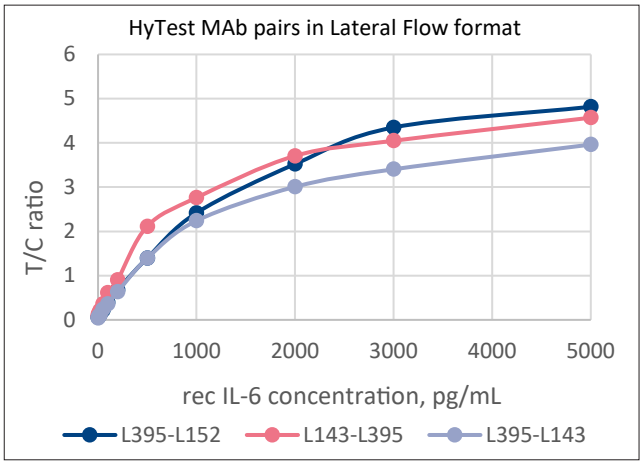

Figure 7.
Calibration curve for selected Hytest MAb pairs in a Lateral Flow assay.

The concentration of serum IL-6 in the clinical samples of patients determined with the Hytest MAb pairs correlate well with the Roche assay, when taken in Lateral Flow format (see Figure 8).

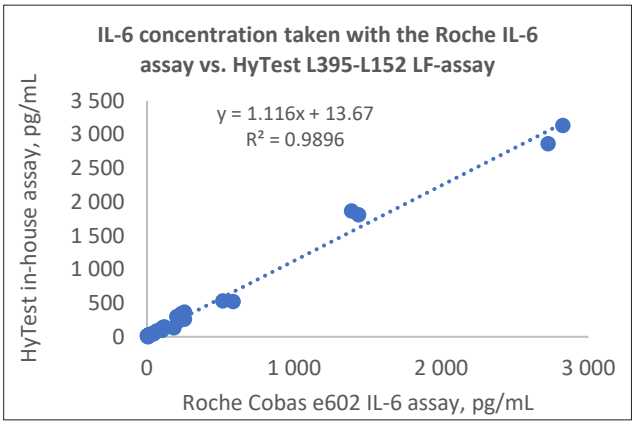

Figure 8.
Determination of IL-6 in the clinical samples of patients with the Hytest MAb pair L395-L152 and correlation to the Roche IL-6 data. Lateral Flow (LF) was used for the Hytest MAb pair.

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Interleukin 6 (IL-6)	4IL6	L106	IgG1	*In vitro*, EIA, LF
		L137	IgG2a	*In vitro*, EIA, LF
		L143	IgG1	*In vitro*, EIA, LF
		L152	IgG1	*In vitro*, EIA, LF
		L395	IgG	EIA, LF, recombinant rabbit antibody
		L519	IgG1	EIA, recombinant chimeric antibody

ANTIGEN

Product name	Cat. #	Purity	Source
Interleukin 6 (IL-6), recombinant	8IL6	>90%	Recombinant

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