

# USE OF sCD40-LIGAND AND PROTEIN S AS BIOMARKERS FOR PREANALYTICAL VARIATIONS OF BIOBANKED SERUM AND PLASMA SAMPLES

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## Background

One of the main issues in biobanking is the establishment of standard operating procedures for specimen collection, preparation and storage, to control for pre-analytical variation. For biological fluids such as serum or plasma, there is currently a lack of sensitive biomarkers for the quality control of cryopreservation conditions. In order for a serum biomarker to be used for QC, it should be ubiquitous and show 100% loss of activity upon inadequate storage conditions and temperature variations.

## Methods

Immunoenzymatic assays were used to assess the stability of the following serum candidate quality control biomarkers, belonging to the cytokine super-family:

C5a	<b>CD40 Ligand</b>	G-CSF	GM-CSF	CXCL1	CCL1	CD54	IFN-g
IL-1a	IL-1b	IL-1ra	IL-2	IL-4	IL-5	IL-6	IL-8
IL-10	IL-12p70	IL-13	IL-16	IL-17	IL-17E	IL-23	IL-27
IL-32a	CXCL10	CXCL11	CCL2	MIF	MIP-1a	MIP-1b	Serpin E1
CCL5	CXCL12	TNF-a	TREM-1				

Chronometric assays were used to assess the stability of the following plasma candidate quality control biomarkers, belonging to the coagulation factors family:

TP	TCA	Fibrinogen	<b>Protein S</b>	Factor II
Antithrombin III	D-dimers	Protein C	Factor VII	Factor VIII

## Results

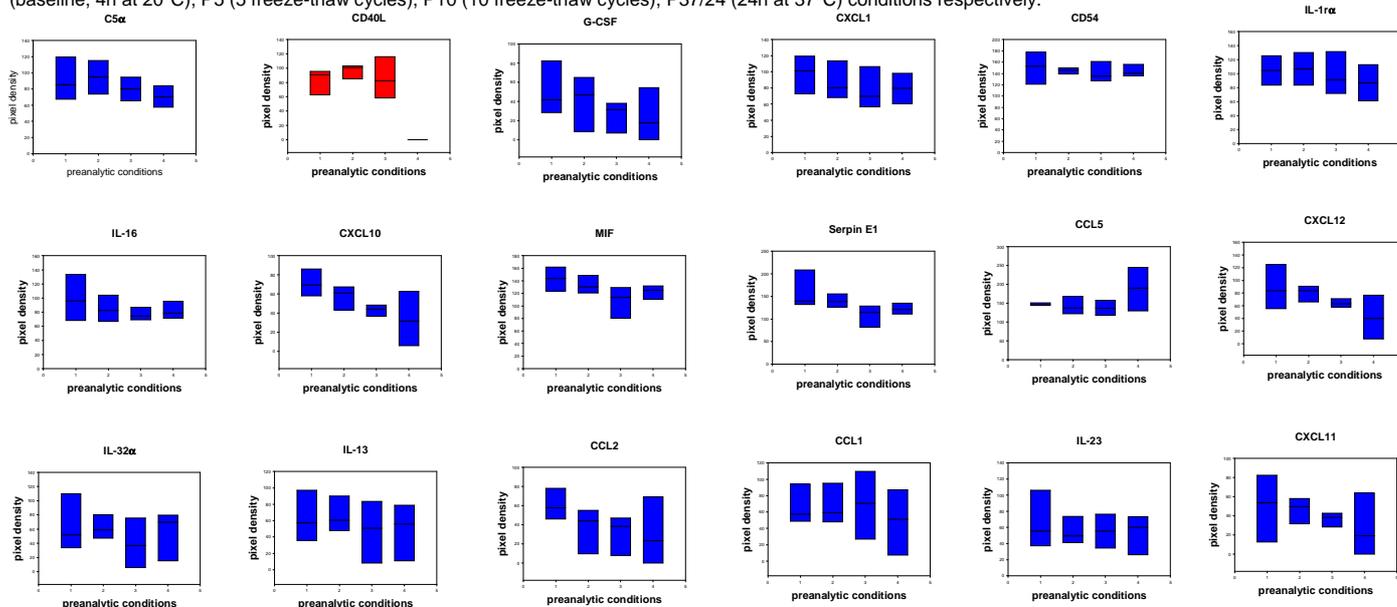
Only CD40 Ligand in serum showed an on-off response to 24h storage at 37°C and protein S in plasma showed a degradation response to 9 years storage at -80°C.

## Conclusions

Biomarkers that have an on-off response to temperature variation could serve as quality indicators for the core processes of biobanking, which are the preparation and storage of biological fluids. The identification of such biomarkers in serum samples is needed. This is the first time, to our knowledge, that such a biomarker is described in serum.

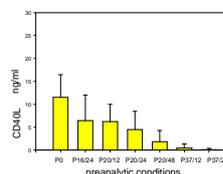
## Figure 1

Pixel densities for ubiquitous cytokines revealed by the cytokine array after 3min (C5a, CD40L, G-CSF, CXCL1, CD54, IL-1ra, IL-16, CXCL10, MIF, Serpin E1, CCL5, CXCL12) or 10min (IL-32a, IL-13, CCL2, CCL1, IL-23) exposures to X-ray film. Medians, and 25<sup>th</sup> and 75<sup>th</sup> percentiles are shown. 1, 2, 3 and 4 correspond to P0 (baseline, 4h at 20°C), P5 (5 freeze-thaw cycles), P10 (10 freeze-thaw cycles), P37/24 (24h at 37°C) conditions respectively.



## Figure 2

Mean values and standard deviations of sCD40L in serum, assessed by ELISA. Serum was subjected to 4h at 20°C (P0), 24h at 16°C (P16/24), 12h at 20°C (P20/12), 24h at 20°C (P20/24), 48h at 20°C (P20/48), 12h at 37°C (P37/12), or 24h at 37°C (P37/24).



## Figure 3

Medians of coagulation parameters at P0 (day of collection) and P9 (after 9-year storage at -80°C); 25<sup>th</sup> and 75<sup>th</sup> percentiles are shown.

