Quantitative Determination of ICAM-1, a Key Regulator of Cell Adhesion and Inflammatory Reactions

1. Introduction

Intercellular Adhesion Molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family (1) and functions as a ligand for the Lymphocyte Function-Associated Antigen-1 (LFA-1), an alpha-beta-complex that is a member of the leukocyte integrin family (2) of cell-cell and cell-matrix receptors. This family consists of the leukocyte adhesion glycoproteins LFA-1 which mediates lymphocyte adhesion, Mac-1 which mediates granulocyte adhesion and p150,95.

ICAM-1 is a single-chain glycoprotein with a polypeptide core of 55kD that can be expressed on non-hematopoietic cells of many lineages such as vascular endothelial cells, thymic epithelial cells, other epithelial cells and fibroblasts and on hematopoietic cells such as tissue macrophages, mitogen-stimulated T-lymphoblasts, germinal center B-cells and dendritic cells in tonsils, lymph nodes and Peyer's patches. ICAM-1 is inducible on fibroblasts and endothelial cells by inflammatory mediators such as IL-1, TNF and IFN-gamma within a few hours and is correlated to the infiltration of lymphocytes into inflammatory lesions (3, 4, 5). ICAM-1 seems to be the initial marker of inflammatory reactions and is expressed prior to, and to a greater extent than is HLA-DR.

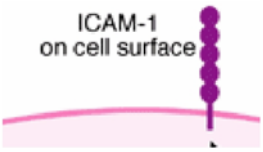

Fig. 1: ICAM-1 is a glycosylated transmembrane protein

The role of ICAM-1 as a disease marker has been demonstrated for a number of different indications and pathological situations:

- **allergic airway inflammation**
- **allergic contact dermatitis**
- **GI-cancer (bladder cancer)**
- **lymphoid malignancies**
- **HIV-1**
- **Malaria**
- **Hepatitis**

2. Materials

- human sICAM-1 Instant ELISA (Bender Medsystems)
- Zenyth 340 Microplate Reader with Evaluation Software
- Adjustable single- and multichannel micropipettes
- Beakers, flasks, cylinders necessary for preparation of reagents

3. Specimen Collection

Cell culture supernatants, human serum, EDTA, or heparinized plasma, spontaneous urine, amniotic fluid, bile, or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive sICAM-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, frozen sera or plasma should be brought to room temperature slowly and mixed gently.

4. Method

Natural human serum samples were applied to **Bender MedSystems Instant ELISA™** microplates. For incubation times and wash cycles refer to the corresponding instruction manual. Samples were measured with a **Zenyth 340** reader at 450nm, reference measurement was taken at 620nm.

5. Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Standard curve is automatically calculated by Zenyth 340 microplate reader. In addition the instrument features 4 different modes of curve fitting:

  - point to point
  - linear regression
  -cubic spline
  - 4 parameter fit

- To determine the concentration of circulating sICAM-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sICAM-1 concentration.

***Samples have been diluted 1 : 10, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 10).**

- It is suggested that each testing facility establishes a control sample of known sICAM-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 2. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed

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	sICAM-1	O.D.	O.D.	C.V.
Standard	Concentration (ng/ml)	(450 nm)	Mean	(%)
1	100	2.293	2.251	2.7
	100	2.208		
2	50	1.668	1.680	1.0
	50	1.692		
3	25	1.176	1.170	0.7
	25	1.164		
4	12.5	0.696	0.701	0.9
	12.5	0.705		
5	6.25	0.395	0.385	3.7
	6.25	0.375		
6	3.13	0.211	0.212	0.7
	3.13	0.213		
7	1.56	0.114	0.116	1.8
	1.56	0.117		
Blank	0	0.023	0.021	
	0	0.018		

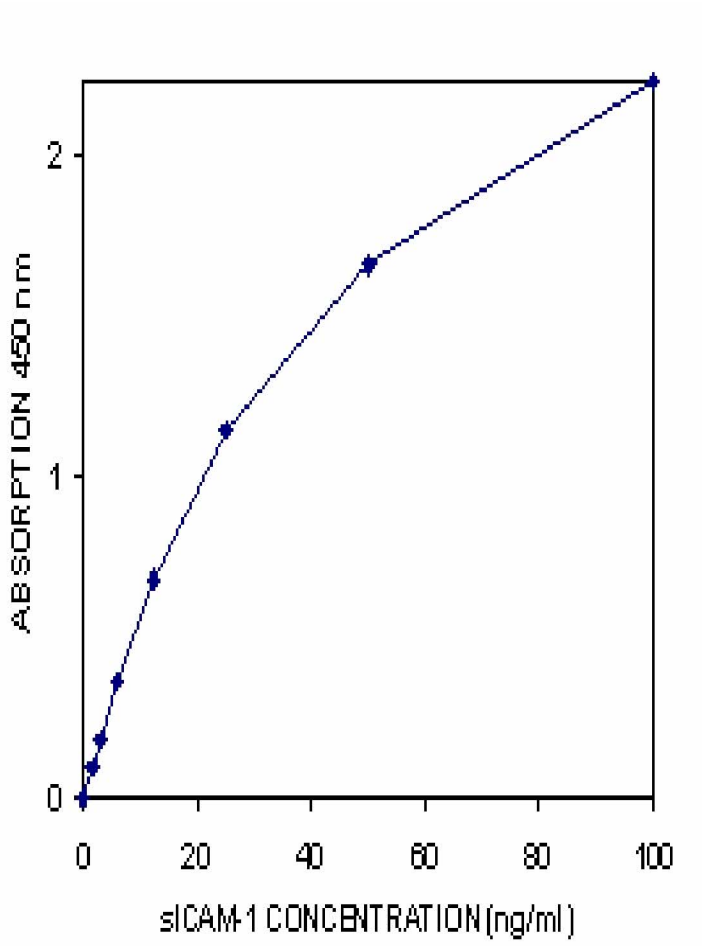

Fig. 2: Shows a representative standard curve, calculated by Zenyth 340 onboard software

6. Results/Summary:

The ideal platform for absorbance measurement for the **Bender MedSystems Instant ELISA**™ turned out to be the **Anthos Zenyth 340 microplate reader.** This absorbance detector allows through its very flexible software a rapid and convenient read out and data processing. **Bender MedSystems' Instant ELISA** technology and the **Anthos reader Zenyth 340** provide a complete solution for busy customers in Biotech and High Throughput laboratories. Both products ensure significant boost of production.

7. Literature

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