

Peptidomics Analysis of Biological Specimens to elucidate Pre-analytical Biases

Harald Tammen, Rüdiger Hess and Paolo Piraino
Digilab Biovision GmbH, Hannover, Germany

1. Introduction

Pre-analytical procedures can alter the outcome of preclinical and clinical studies. In particular sample collection and specimen preparation can alter the validity of results obtained by modern multiplex assays (e.g. LC-MS). Quality assessment of collected samples is crucial to avoid systematic biases. Here we introduced the application of peptidomics to grade samples with respect to pre-analytical biases. The analysis of native peptides (Low Molecular Weight [LMW-] proteome) in biological specimens is primarily achieved by chromatographic methods coupled to diverse forms of mass spectrometry. Peptides as products of proteolytic cleavage events exhibit a close connection to protease activity. Increased or altered activity of proteases during sample collection, specimen generation, sample storage and processing is mirrored by alterations in abundance of specific peptides. To demonstrate this approach we analyze different blood specimens to elucidate biological events occurring during the pre-analytical phase by peptidomics and qualify the specimens by sample grading.

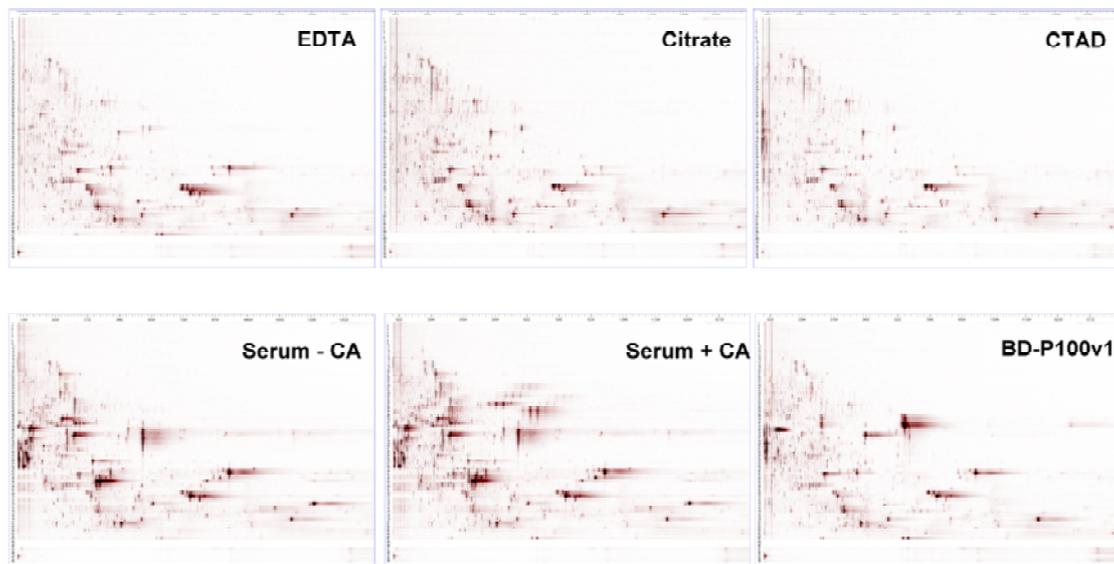


Figure 1: Peptide displays from blood specimens

Peptide displays of the 6 blood specimens (EDTA plasma, citrate plasma, CTAD plasma, serum with and without clot activator and P100v1). The x-axis displays the mass-to-charge ratio, the y-axis is determined by the retention time on the RP-HPLC. The signal intensity is depicted by color saturation. Between 3,000 and 7,000 signals are visualized per peptide display.

2. Blood Withdrawal and Specimen Collection

Healthy adult individuals were enrolled into these studies after they provided written informed consent and the local Ethics Committee at the Hanover Medical School approved the protocol. The blood samples were collected from the cubital vein into blood collection tubes (EDTA-Plasma, Citrate, CTAD [citrate, theophylline, adenosine, dipyridamole] Serum with and without clot activator; P100: BD-P100v1). Immediately after withdrawal, plasma was separated from cells by a 2-step centrifugation (1st: 10 min at 2000 x g at room temperature (RT); 2nd: 15 min 2500 *g at RT). Plasma aliquots were transferred into 2 mL Eppendorf vials. Serum was obtained after coagulation of blood for approx. 1 h. The collection tube was centrifuged for 10 min at 4°C at 2,000 x g. The serum was separated from the clot and stored at -80°C until analysis. Preparation of P100 samples was according to instruction provided by the manufacturer.

3. Peptidomics Analysis

To assess the quality of a given sample an aliquot of the respective sample is analyzed via peptidomics to obtain peptide profiles: briefly, peptides were extracted and separated in 96 fractions by liquid chromatography. An aliquot of each fraction was subjected to MALDI mass spectrometry. After data acquisition data was visualized by applying the concept of peptide displays. Visual representation provides a vivid interface for the data analyst, helps to assess sample as well as process related quality aspects (e.g. performance of separation, m/z de-calibration, oxidation or presence of contaminants), and supports the process of knowledge extraction. Data analysis is carried out to retrieve significant mass spectrometric signals representing surrogate peptides for pre-analytical events. By analyzing the abundance of these surrogate peptides (n=77) distinct biological events (coagulation (23), kallikrein activation (18), complement activation (8), hemolysis (3) and presence of residual platelets (25)) and their significance can be attributed to each sample to ensure comparability between samples.

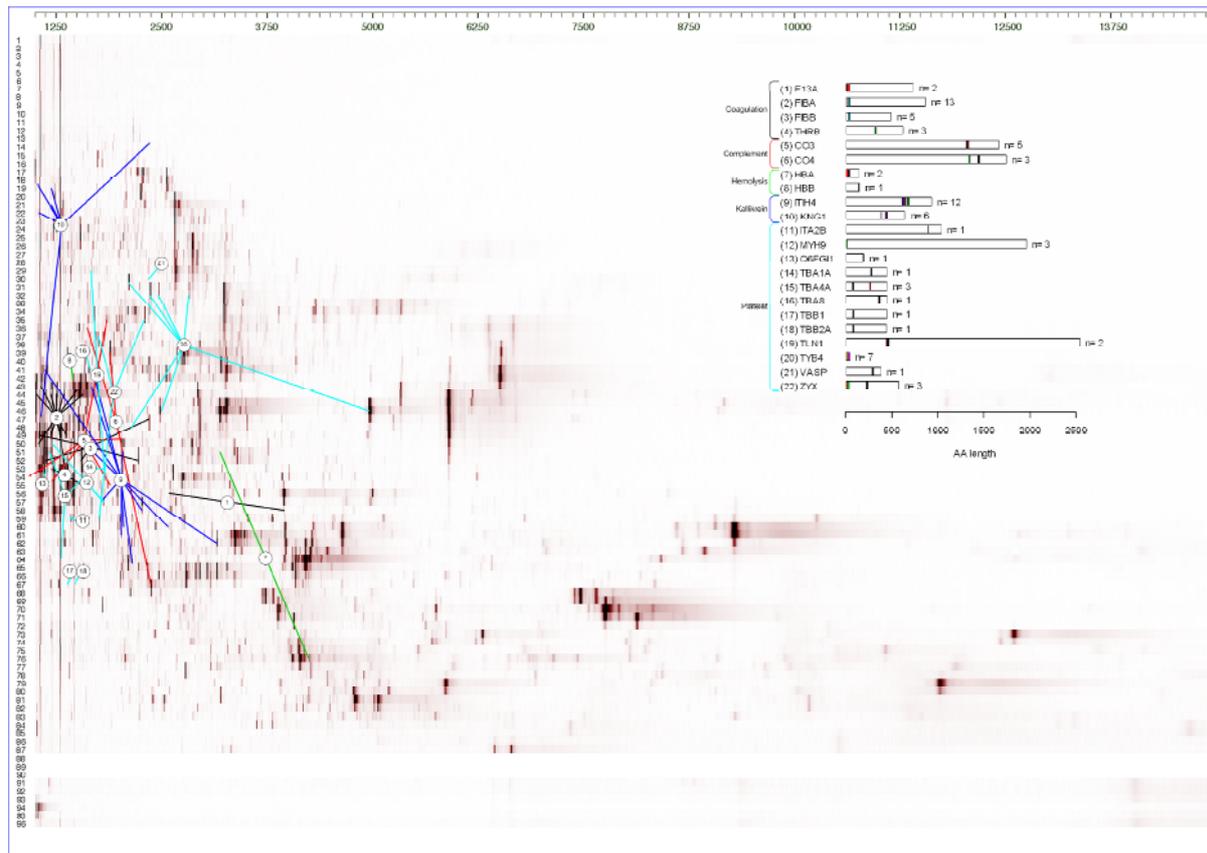


Figure 2: Distribution of surrogate peptides

The figure depicts a mean peptide display of all specimens analyzed. Peptides derived from a distinct precursor protein are visualized by patterns of straight lines connected to a central node. The number inside the node refers to the precursor protein (cf. inlet).

Inlet: Each bar represents one protein precursor. The peptides which derived from this protein are depicted as colored bars. The numbers in parenthesis refer to the network node on the left. The numbers right to the columns refer to the number of peptides derived from the corresponding precursor protein.

4. Grading of Blood Specimens

To demonstrate this approach we analyze different blood specimens to elucidate biological events occurring during the pre-analytical phase by peptidomics and qualify the specimens by sample grading. Each specimen shows a distinct elevation of surrogate peptides used for sample grading. EDTA, Citrate and CTAD plasma exhibit a more similar pattern in comparison to Serum and P100v1. The latter specimens show signs of cell lysis, coagulation and activation of kallikrein. An in-depth analysis of EDTA, Citrate and CTAD plasma also demonstrate pronounced differences between these specimens.

The main purpose of this study was to demonstrate the massive impact on generation of artificial ex-vivo generated peptides within the different specimens. The foremost application of this approach would be the characterization of sample quality inside study populations to determine possible pre-analytical biases.

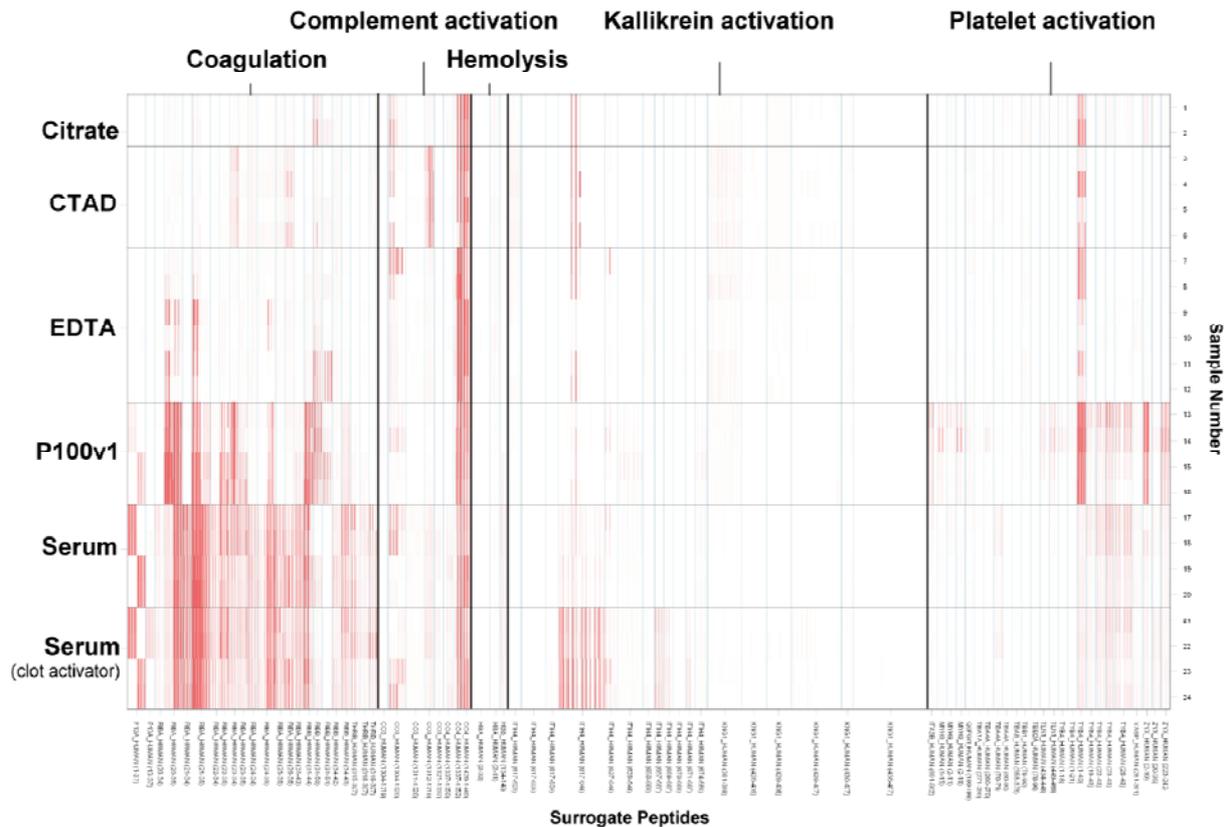


Figure 3: Quality Grading Chart

Peptide surrogate markers were exported and analyzed according to distinct biological events. The left axis depicts the human blood specimen, the bottom axis the surrogate peptide (precursor abbreviation and amino acid range) and the top axis the biological event represented by groups of surrogate peptides. It is clearly visible that Serum and P100v1 exhibit significant signs of coagulation and platelet activation. Additionally the clot activator in serum samples lead to an activation of plasma kallikrein (surface activated).

5. Conclusion

The determination of sample quality is an important issue to ensure validity of results. Particularly in the field of multiplex assays it is a prerequisite for effective study outcomes. Sample grading by Peptidomics is a versatile tool to qualify biological specimens. The analysis of pre-analytical biases allow for characterization of samples, detection of outliers and recognition of erroneous sample handling procedures.

6. References

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7. Contact

Leif Honda
120 Cedar Street
Canton, MA 02021
Phone:(781) 575-0310
www.digilabglobal.com
lhonda@digilabglobal.com