

Stabilization of Cytokine Biomarkers in Human Blood

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Abstract

The most facile diagnostic and toxicological tests measure changes of biomarkers in blood. Whole blood remains the gold standard sample for diagnostics due to the ease of quantitative measurement and serial monitoring of biomarkers in blood. The diversity of analytical instruments for evaluating biomarkers in blood also allows rapid analytical measurements of blood samples. Less invasive and analogous techniques for the analysis of similar biomarkers in other body fluids are also gaining broader use but blood still remains the most reliable source of biomarkers for the diagnosis of disease. However, blood-based diagnostics are limited by storage and transportation logistics between the clinic and the laboratory. This problem is not limited to blood collection and shipping from remote or resource limited locations. Research focused on the discovery of new biomarkers, such as certain nucleic acids, cytokines, and cells, are hampered by their short half-life, reducing their diagnostic and research applications even in modern research and testing facilities. Thus, methods for stabilizing blood will extend the reach of diagnostic approaches by stabilizing these markers long enough to retain their diagnostic value. Biomatrixa has developed a number of formulations to stabilize whole blood at the point of specimen collection. In this study, our results indicate the effectiveness of these Biomatrixa stabilizers. These formulations can extend the lifetime of many unstable markers without affecting the stability of naturally stable markers. Application of Biomatrixa's stabilizers will create a greater and more robust testing window for many of valuable classes of biomarkers.

Introduction

Our aim is to develop stabilizers that maintain the integrity of whole blood and separated components as assay-ready samples at ambient temperatures. The preserved blood samples offer an alternative to cold-chain logistics and provide reliable recovery and analysis of blood components including cytokines, peptides, and whole cells including their nucleic acid content, sugars, small molecules, and other biomarkers. In this study we have evaluated a set of our proprietary stabilizers on stability of a panel of cytokines typically found in human blood. The cytokines were added exogenously to fresh human plasma following mixing with various stabilizers. The blood/mixture was then stored at room temperature. The stabilities of cytokines were examined over a 14-day period. Our overall results show that the stabilizers protected 27 of 29 cytokines up to 79% of the original concentrations. Here, we show an example of one of the stabilizers on the stabilities of Eotaxin, IFN γ , IL-13, IL-2, IL-1 β , and TNF α in human blood.

Result

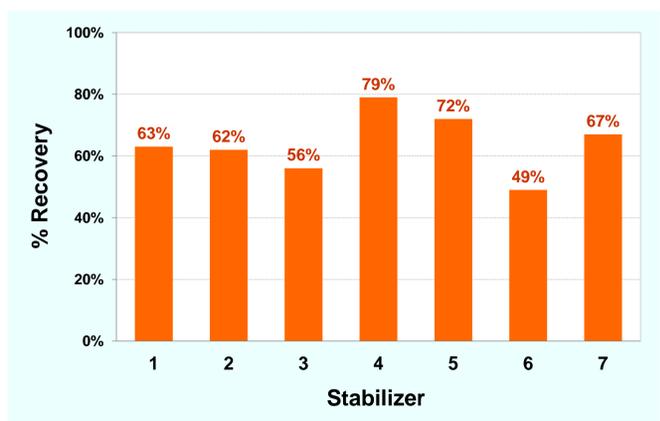


Figure 1. Overall performance of various stabilizers on cytokine stabilities in human plasma. The average effects of the indicated stabilizers on the recovery of all cytokines over a 14-day period is indicated as % recovery which was normalized to the non-protected control values taken at time zero. The result indicates that stabilizer #4 achieves the highest cytokine recovery over this time period.

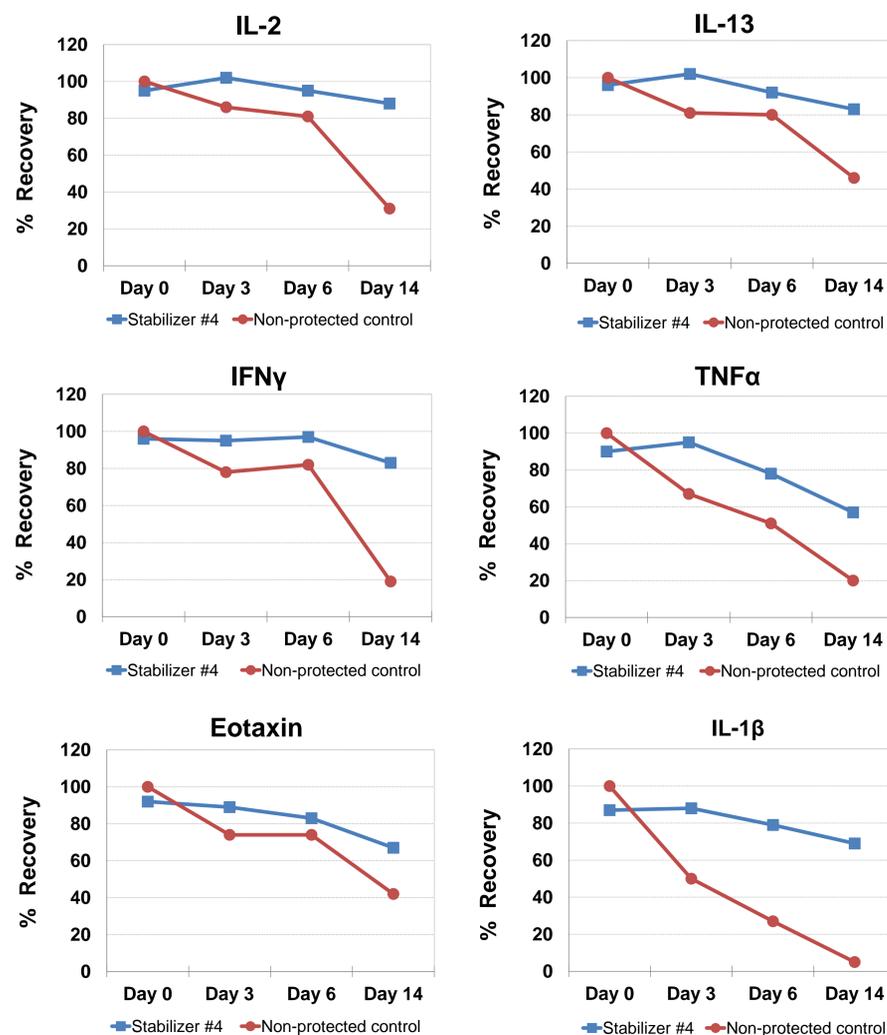


Figure 2. Performance of stabilizer #4 on stability of cytokines in human plasma. Cytokines were introduced into human plasma, and mixed with the stabilizer as described in Material & Method. Quantification of cytokines was performed via the Luminex MagPix system at the indicated time points. The raw assay values were normalized to the non-protected control values taken at time zero.

Material & Method

We evaluated 7 stabilizers for their abilities to stabilize a panel of cytokines typically found in human blood with a series of "spike-in" experiments.

A panel of cytokine standards was purchased from EMD-Millipore designed for use with the Luminex™ Magpix system. Human blood was collected from healthy volunteers and the plasma was isolated using standard procedures. The plasma was aliquoted and frozen at -80°C until use. 6 μ L of 400 pg/ml cytokine standard solution was added to 69 μ L of plasma and combined with 75 μ L of a stabilizer formulation to achieve a stabilizer vs. plasma is 1:1 (vol:vol). The mixture of stabilized plasma was then stored at room temperature for 0, 3, 6, 14 days. Quantification of cytokines was performed using the Luminex™ MagPix system. Controls consisted of plasma mixed with buffer without stabilizers. All relative concentrations reported were standardized to these controls. The raw values were normalized to the non-protected control values taken at time zero.

Summary

We have evaluated a set of our proprietary stabilizers on stability of a panel of cytokines typically found in human blood. Our overall results show that stabilizers protected cytokines up to 79% of the original concentrations. Among the stabilizers that we examined, stabilizer #4 showed the best protection on cytokines in human blood. As shown in Figure 2, the stabilities of cytokines treated with the stabilizer #4 were significantly higher than the unprotected controls at zero time point. This stabilizer conferred good stability for up to 14 days at room temperature in cytokines such as IL-2 (88% vs. 31%), IL-3 (83% vs. 46%), IFN γ (83% vs. 19%), TNF α (57% vs. 20%), Eotaxin (67% vs. 42%), IL-1 β (69% vs. 5%).