

Effects of Blood Sample Handling Procedures on Measured Cytokine and Chemokine Concentrations in Human Serum and Plasma

Diplomarbeit
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Christian Doppler Laboratory for Cardiac and Thoracic
Diagnosis and Regeneration

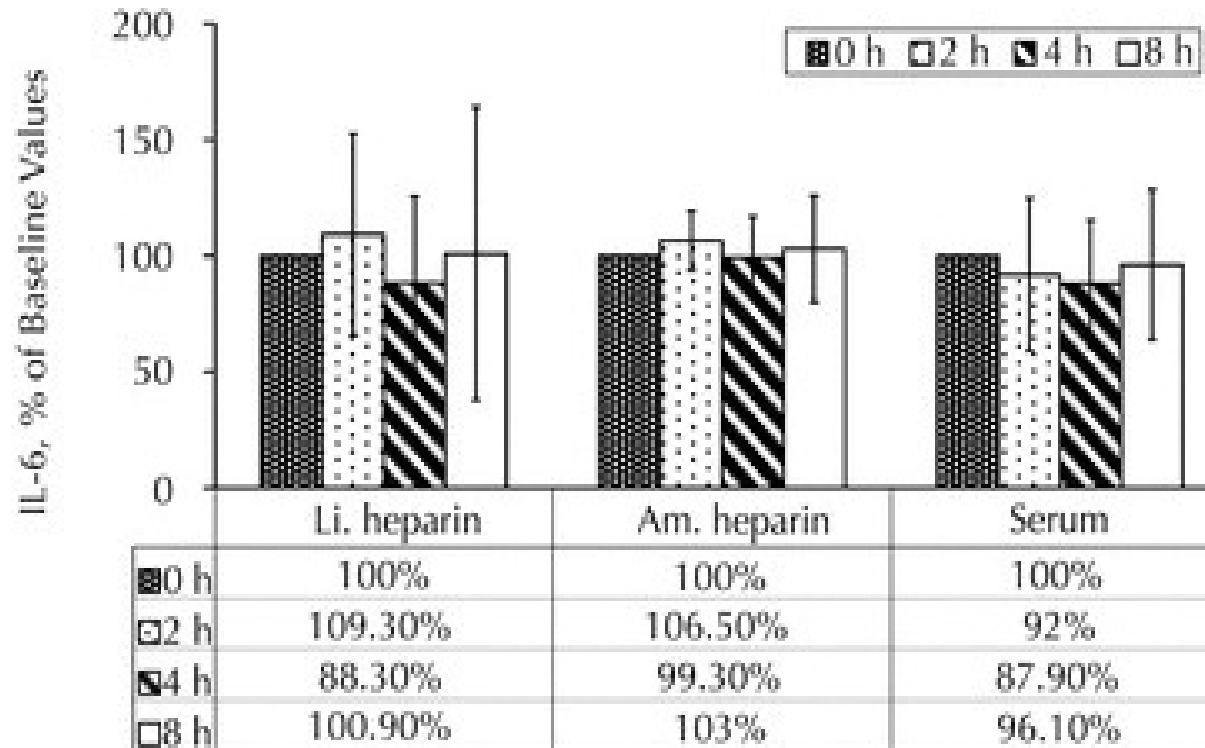
unter der Anleitung von
Ass. Prof. Univ.-Doz. Dr. Hendrik Jan Ankersmit

Introduction

- Cytokines are cell-signaling proteins that are secreted by numerous cell types
- Cytokine concentration patterns are altered in a variety of diseases
- Cytokine measurement in body fluids is popular in research studies as well as in clinics
- Inconsistent data are available characterizing preanalytic factors influencing cytokine concentrations in human serum and plasma samples



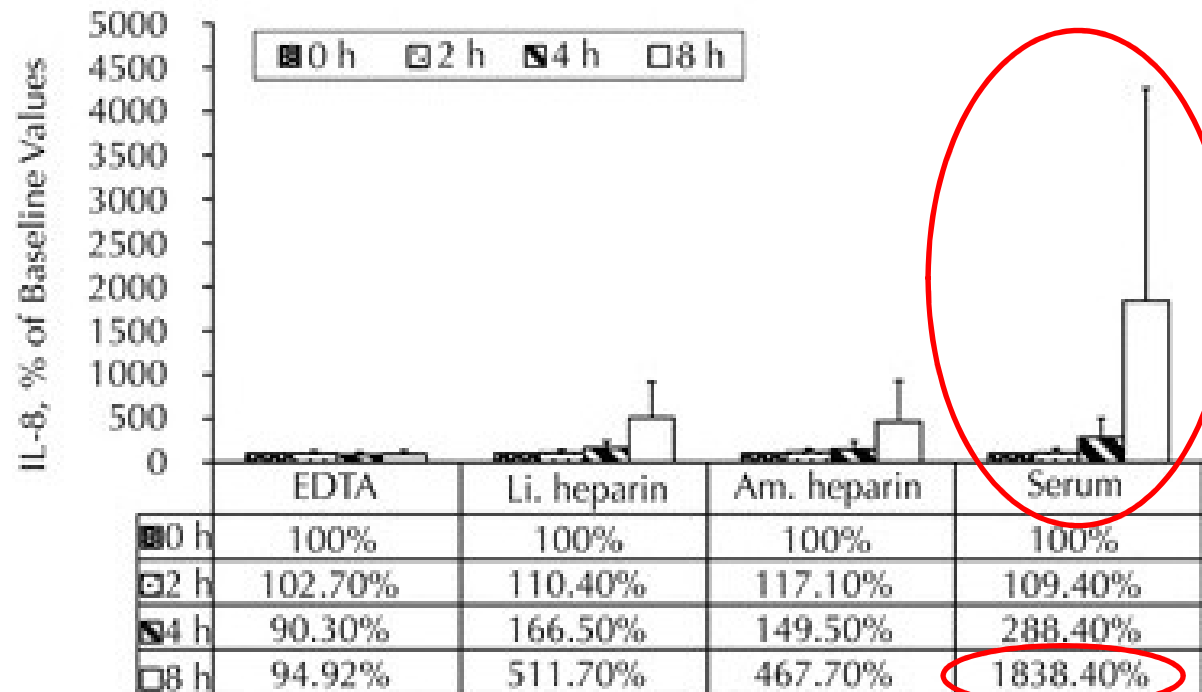
Background



IL-6 concentrations at 0 h, 2 h, 4 h, 8 h storage at 24°C



Background



F IL-8 concentrations at 0 h, 2 h, 4 h, 8 h storage at 24°C



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Technical Background



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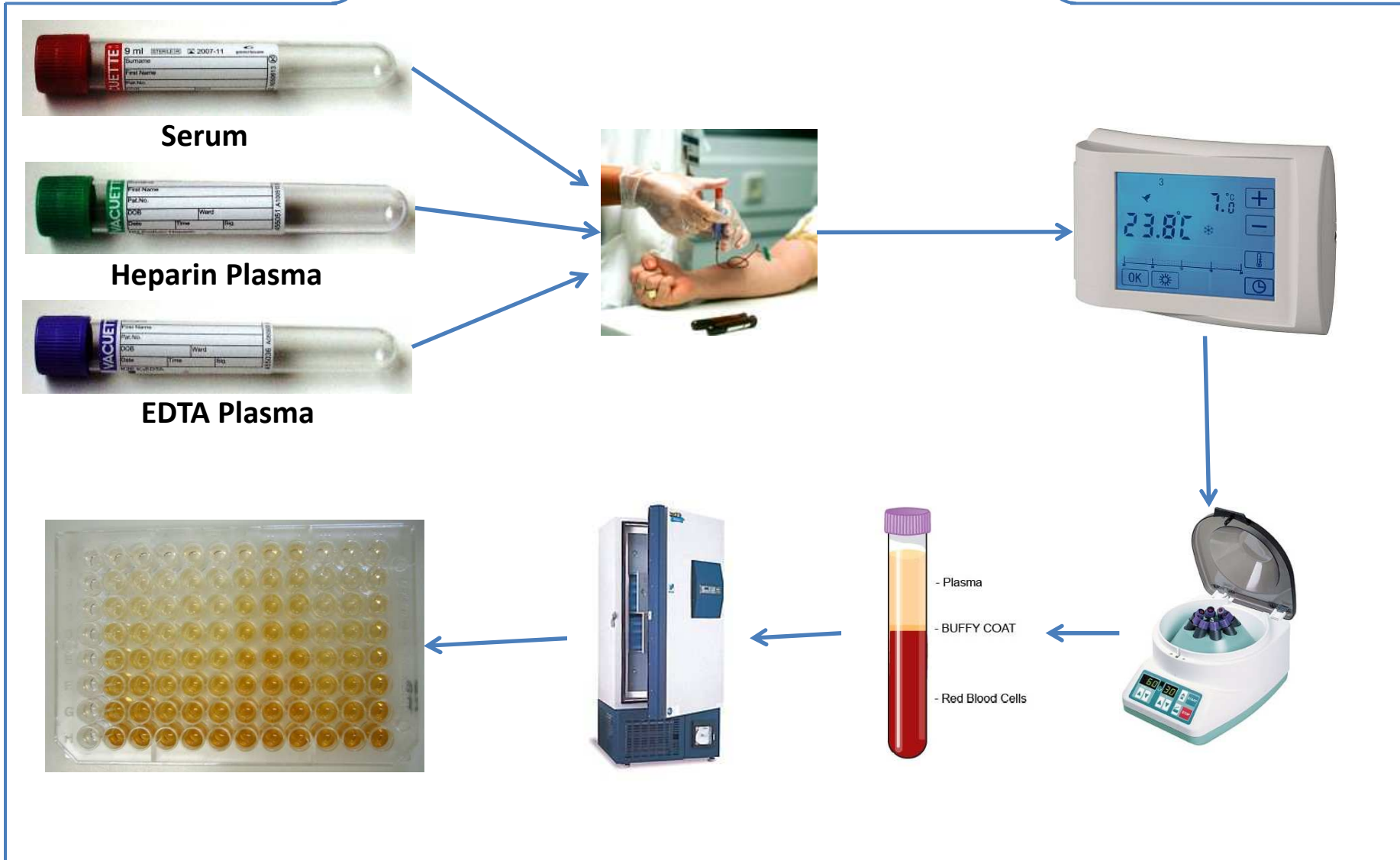
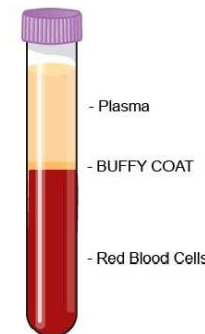
Serum



Heparin Plasma



EDTA Plasma



- Requirements on cytokine measurement systems
 - Specificity
 - Sensitivity
 - Simplicity
 - Reliability
 - Cost
 - Time



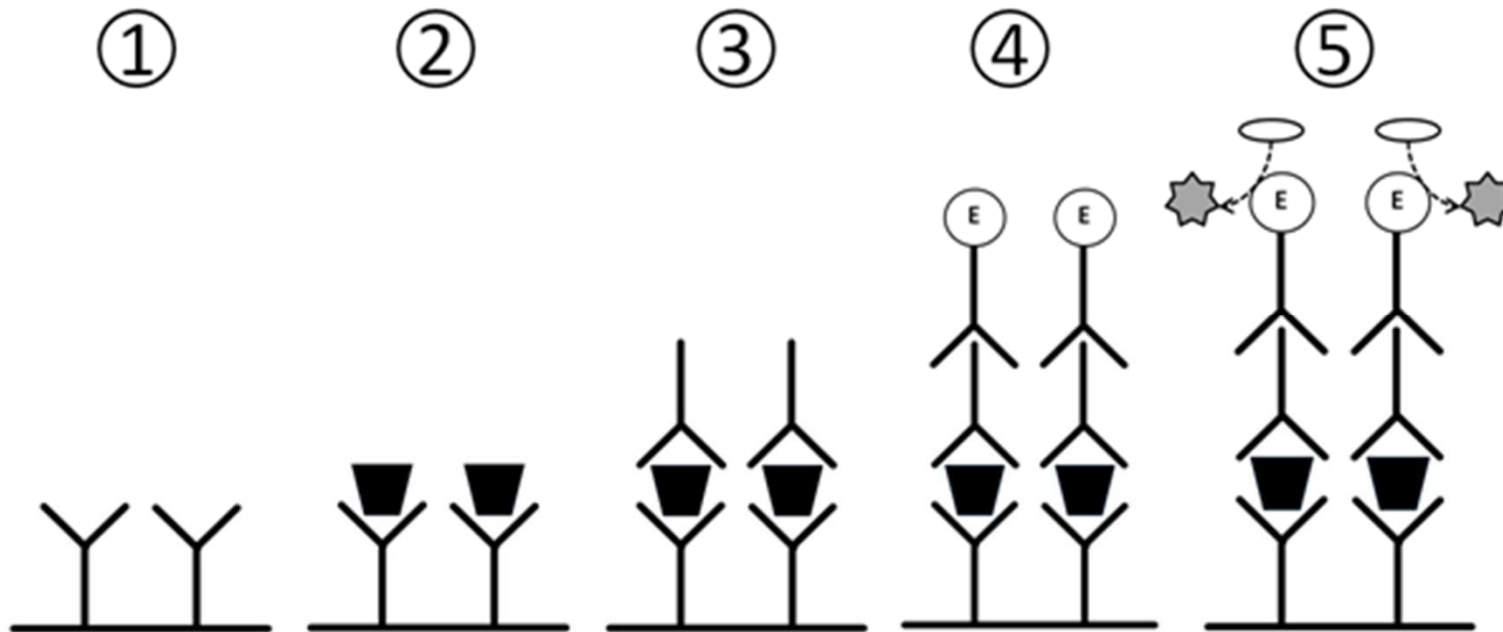
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Enzyme-Linked Immunosorbent Assay (ELISA)



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OPEN ACCESS Freely available online



Levels and Determinants of Inflammatory Biomarkers in a Swiss Population-Based Sample (CoLaus Study)

Pedro Marques-Vidal^{1*}, Murielle Bochud¹, François Bastardot², Thomas Lüscher³, François Ferrero⁴, Jean-Michel Gaspoz⁵, Fred Paccaud¹, Adrian Urwyler⁶, Roland von Känel⁷, Christoph Hock⁸, Gérard Waeber², Martin Preisig⁹, Peter Vollenweider²

Biomarkers, 2010; 15(2): 140–148

informa
healthcare

ORIGINAL ARTICLE

Stability and reproducibility of simultaneously detected plasma and serum cytokine levels in asymptomatic subjects

Fatemeh Saberi Hosnijeh^{1,2}, Esmeralda J. M. Krop¹, Lützen Portengen¹, Charles S. Rabkin³, Jakob Linseisen⁴, Paolo Vineis⁵, and Roel Vermeulen^{1,6}

Aims of the study

- Does sample handling procedures alter measurable cytokine concentrations?
- Differences between serum and plasma tubes?
- Triggers for cytokine release in vitro?

Measured Cytokines

- IL-8
- GRO- α
- ENA-78
- GCP-2
- MCP-1
- IL-6
- IL-1 β
- TNF- α
- TGF- β
- VEGF
- IL-1RA

IL-8



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Experimental design



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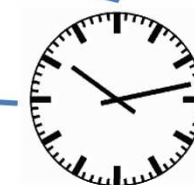
Serum



Heparin Plasma



EDTA Plasma



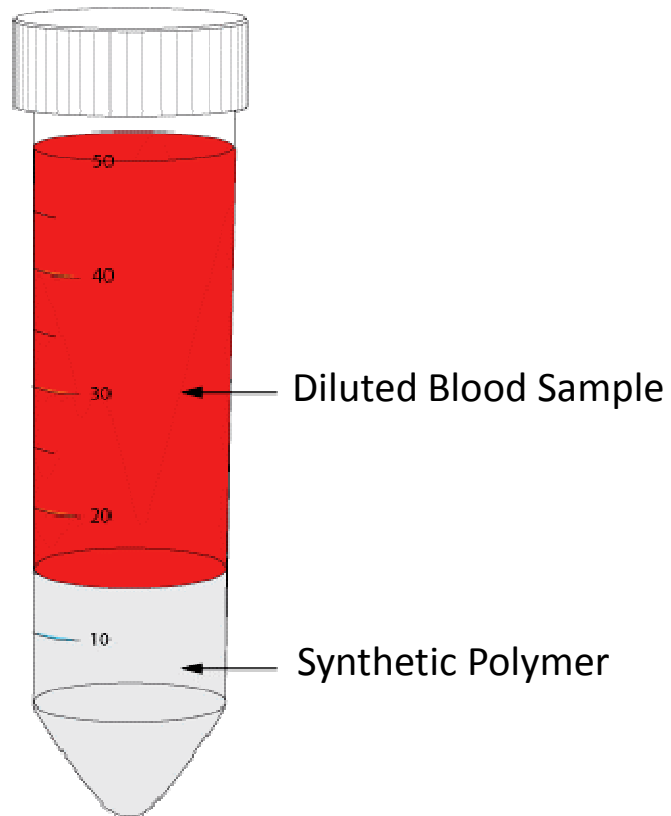
- Plasma
- BUFFY COAT
- Red Blood Cells



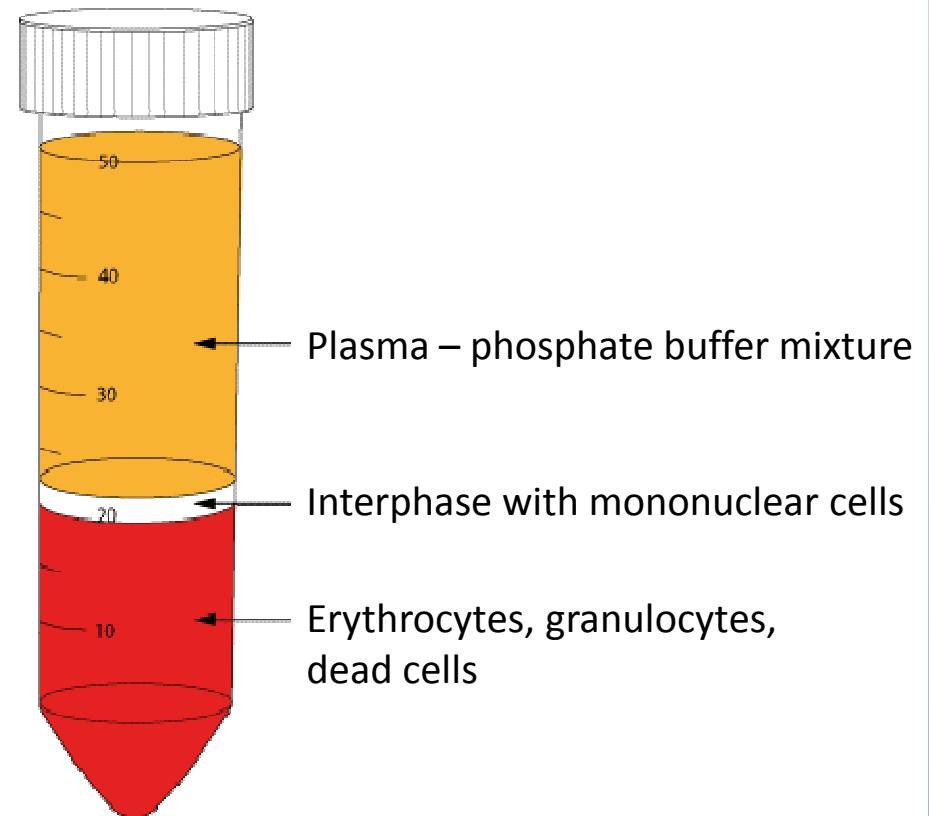
n = 7

PBMC experiment

before centrifugation



after centrifugation





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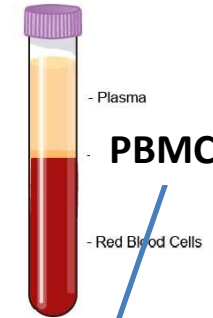
PBMC experiment



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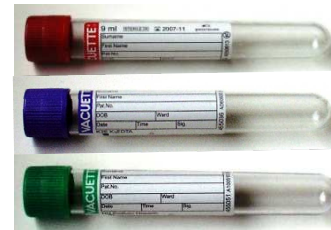
Heparin Plasma



- Plasma

PBMC

- Red Blood Cells

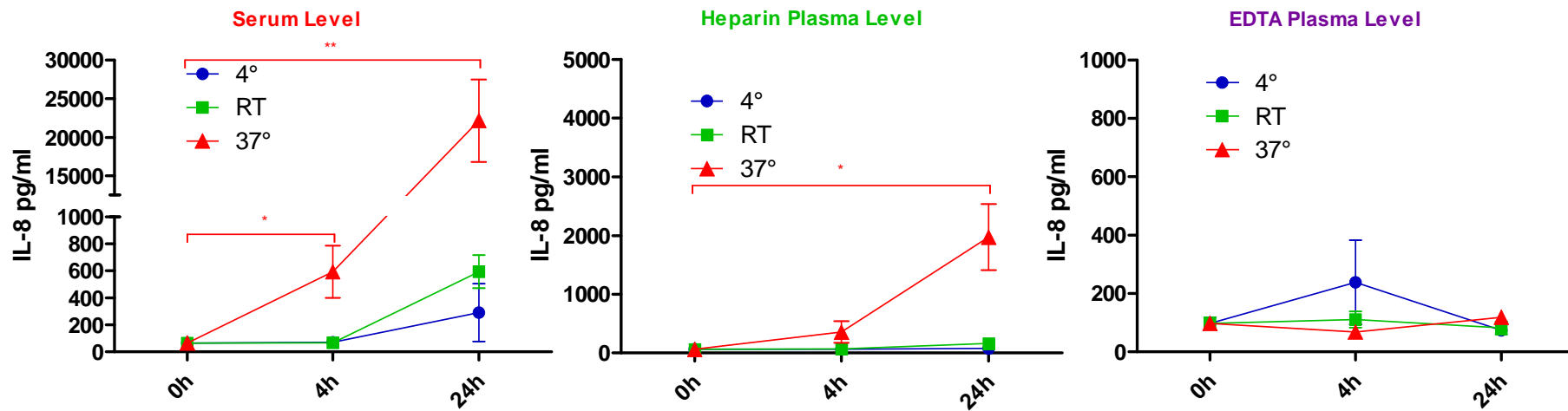


human Fibrin



n = 8-12

Whole blood experiment

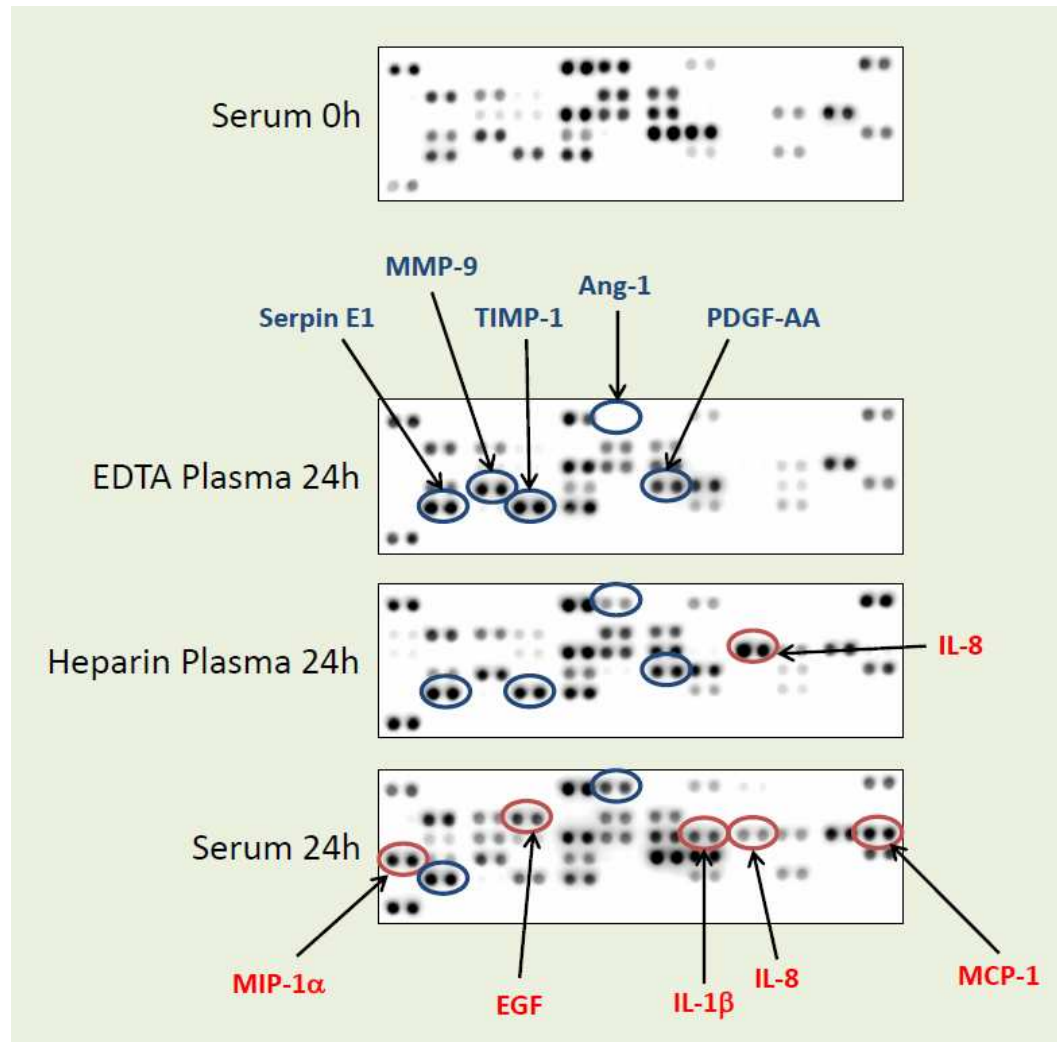


* = p < 0.05; ** = p < 0.01

n = 7



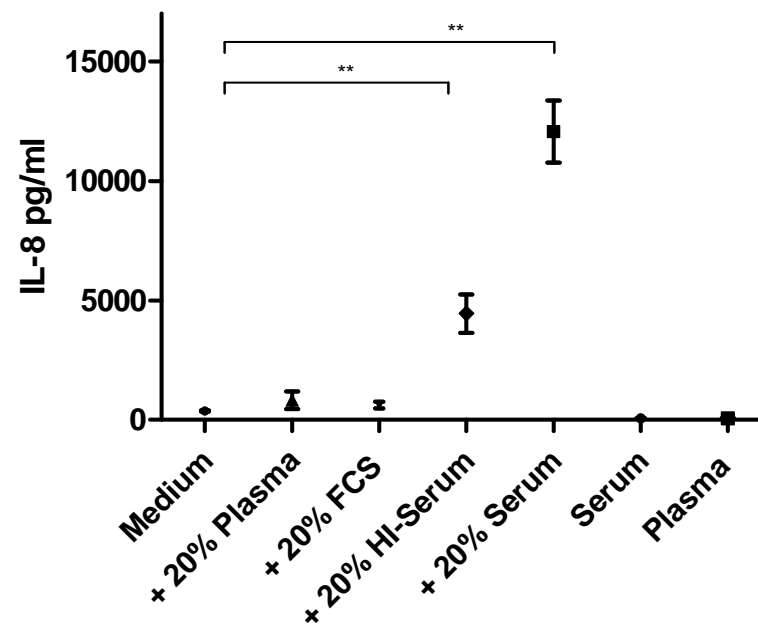
Results



n = 7

Results

In vitro experiments



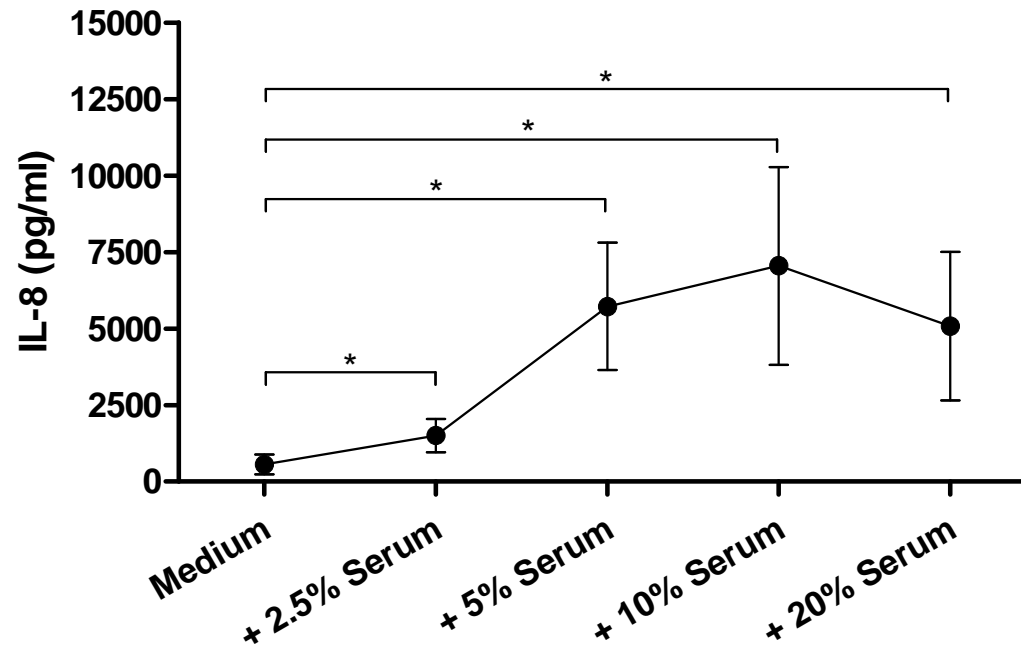
PMBC supplemented with: autologous plasma; FCS = fetal calf serum, HI-Serum = heat inactivated serum; autologous serum, Serum and Plasma without PMBC

* = $p < 0.05$; ** = $p < 0.01$

n = 12

Results

In vitro experiments



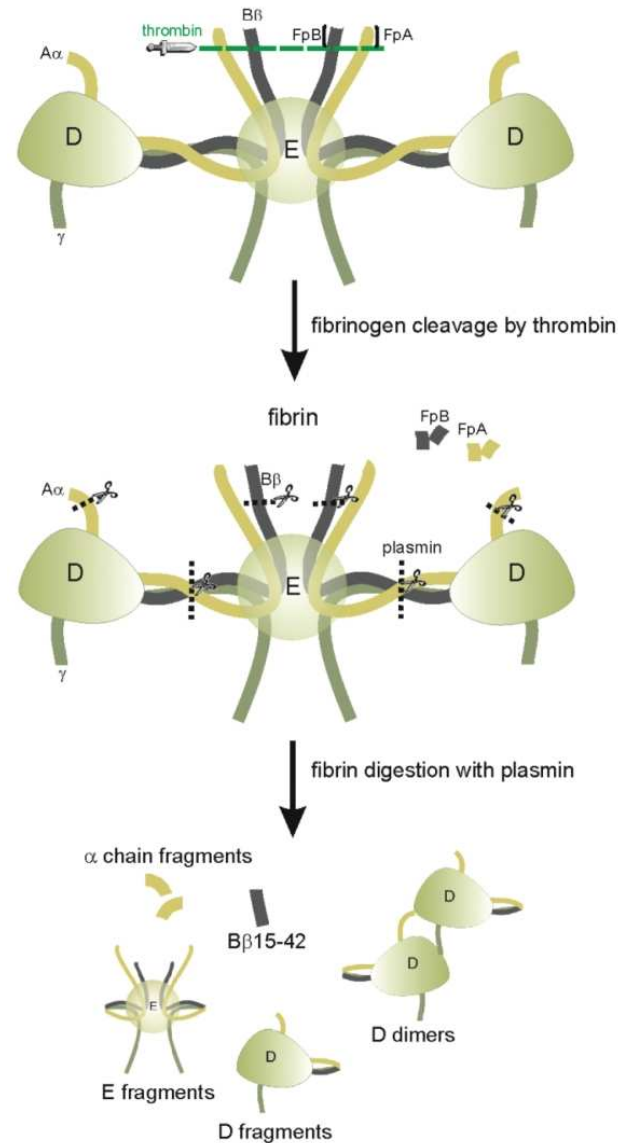
PBMC supplemented with increasing dosis of autologus serum

* = $p < 0.05$; ** = $p < 0.01$

n = 8



Fibrin(ogen)

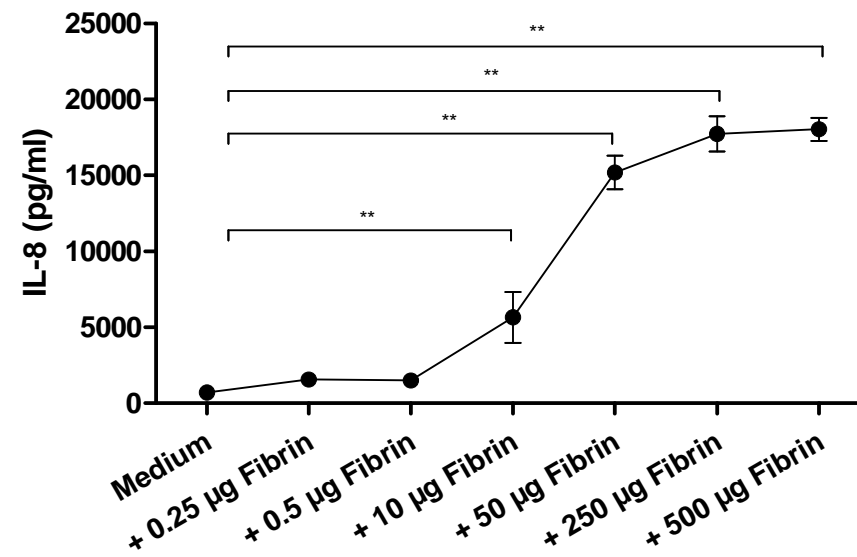


Fibrinogen:

- Soluble Dimer: 340 kDa
- α chain: 63 kDa
- β chain: 56 kDa
- γ chain: 47 kDa

Results

In vitro experiments



PBMC supplemented with increasing dosis of fibrin

* = $p < 0.05$; ** = $p < 0.01$

n = 8

Conclusion

- Immediate processing of plasma and serum is essential
- Non-centrifuged samples should be stored at 4°C
- EDTA plasma tubes seem to be most usable for stability reasons
- Comparison between serum and plasma concentrations has to be interpreted critically
- Fibrin stimulates cytokine secretion of PBMC

Special thanks

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