

Advanced Single Molecule Detection Accelerating Biomarker Development Utilizing Cytokines through Ultrasensitive Immunoassays

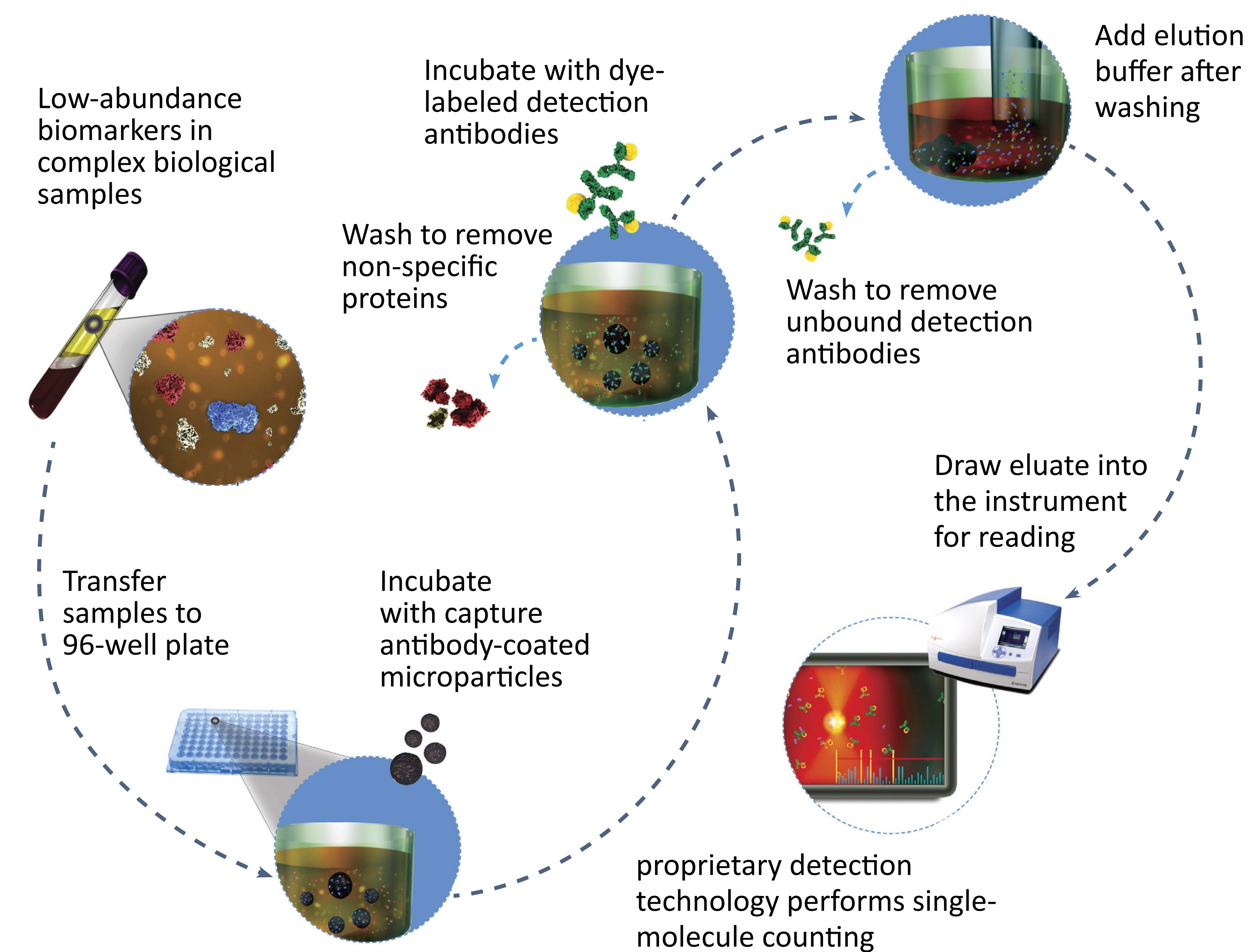
J. Greenberg¹, V. Furer¹, Q.A. Lu², R. Ramirez², S. Abramson¹, M. Attur¹, J. Hsu², L. Zieske² ¹NYU Hospital for Joint Diseases, New York, NY. ²Singulex, Inc., Alameda, CA
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Abstract & Introduction

Dysregulation of the cytokine system has been implicated for many autoimmune and inflammatory disorders, such as rheumatoid arthritis, asthma, psoriasis and cardiovascular disease. Recent research has shown that detection and monitoring of cytokine concentrations can provide insights into disease progression and thus, cytokines have become attractive biomarkers and candidates for targeted therapies. However, due to the low-abundance of these circulating markers, such as IL-17A, obtaining robust measurements in clinical samples has been difficult. To address this critical need, Singulex developed a single-molecule counting technology with increased detection sensitivity to help shed light on biomarker verification and validation programs. The patented Erenna[®] Immunoassay system, which includes uniquely optimized immunoassays, offers sub-picogram per mL resolution at an improvement of 1-3 fold over standard ELISAs. This sensitivity improvement helps minimize undetectable samples that could otherwise delay or derail clinical studies. Here we present case studies demonstrating how the use of the Erenna Immunoassay System has provided critical insights toward improving the clinical utility of biomarkers and accelerating the development of novel therapies for treating inflammatory diseases.

Technology

Figure 1. Singulex high-definition immunoassays, coupled with the ultrasensitive Erenna Immunoassay System and proprietary control software Sgx link[™], accelerate biomarker research by providing sub-picogram/mL biomarker detection across multiple disease areas.



Singulex[®]

1500 Harbor Bay Parkway, Suite 200.
Alameda, CA 94502
T: 510.995.9040
F: 510.995.9018
E: www.singulex.com

Case Study: RA vs Healthy

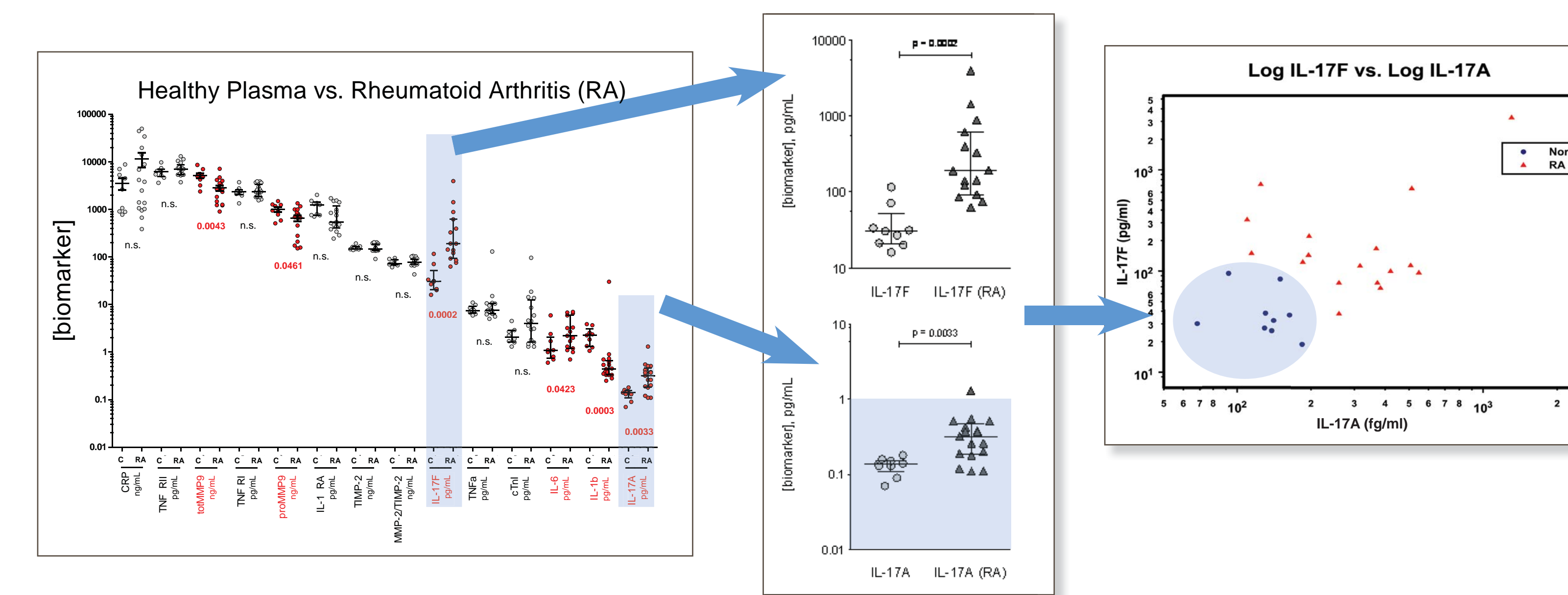


Figure 1. A multi-marker analysis of rheumatoid arthritis patients (RA) vs age-gender matched healthy volunteers (HV) was conducted using the Singulex technology. The study identified markers with significant concentration differences between healthy and disease populations using a limited population set. In particular, Singulex provides the sensitivity and quantifiability needed to accurately measure baseline IL-17A concentrations in HV, as well as minute elevations in RA. In conjunction with data from IL-17F, this dual marker differentiation powered by Singulex allows for 100% specificity for RA patients in this study.

Table 1. Analytical performance of assays presented in this study. Singulex assays offer greater sensitivity and lower sample volume requirement, allowing baseline biomarker levels to be accurately measured in age-gender matched healthy volunteers.

Biomarker	ctrl	IL-1β	IL-1 RA	TNF RI	TNF RII	TNFα	IL-6	IL-17F	IL-17A	IL-17A/F heterodimer	HS-CRP	VEGF-a	TIMP-2	MMP-2	MMP-2/TIMP2	totMMP9	proMMP9
LLoQ (pg/mL)	0.8	0.1	2	0.2	0.02	0.02	0.01	1.5	0.05	0.15	0.5	0.09	0.6	2	4	1.6	8
AVG [Analyte] in HV Plasma (pg/mL)	2	2	1220	2503	6488	8	2	42	0.2	0.8	3101	20	156	55	77	5091	997
Sample Volume Needed (μL)	20	20	8	1	1	5	5	10	100	100	1	5	1	2	4	1	1

Conclusion:

- Current detection methods for quantifying low-abundance biomarkers, such as IL-17A, IL-17F, and IL-17AF are inadequate due to lower limits of quantification.
- The patented single-molecule counting technology provided by Singulex offers superior sensitivity. With lower limits of quantification, only a minute sample volume is required for the determination of biomarker concentration at baseline. This allows precious sample to be conserved from high-profile clinical study specimens, and multi-panel studies to be performed.

Case Study: RA vs OA

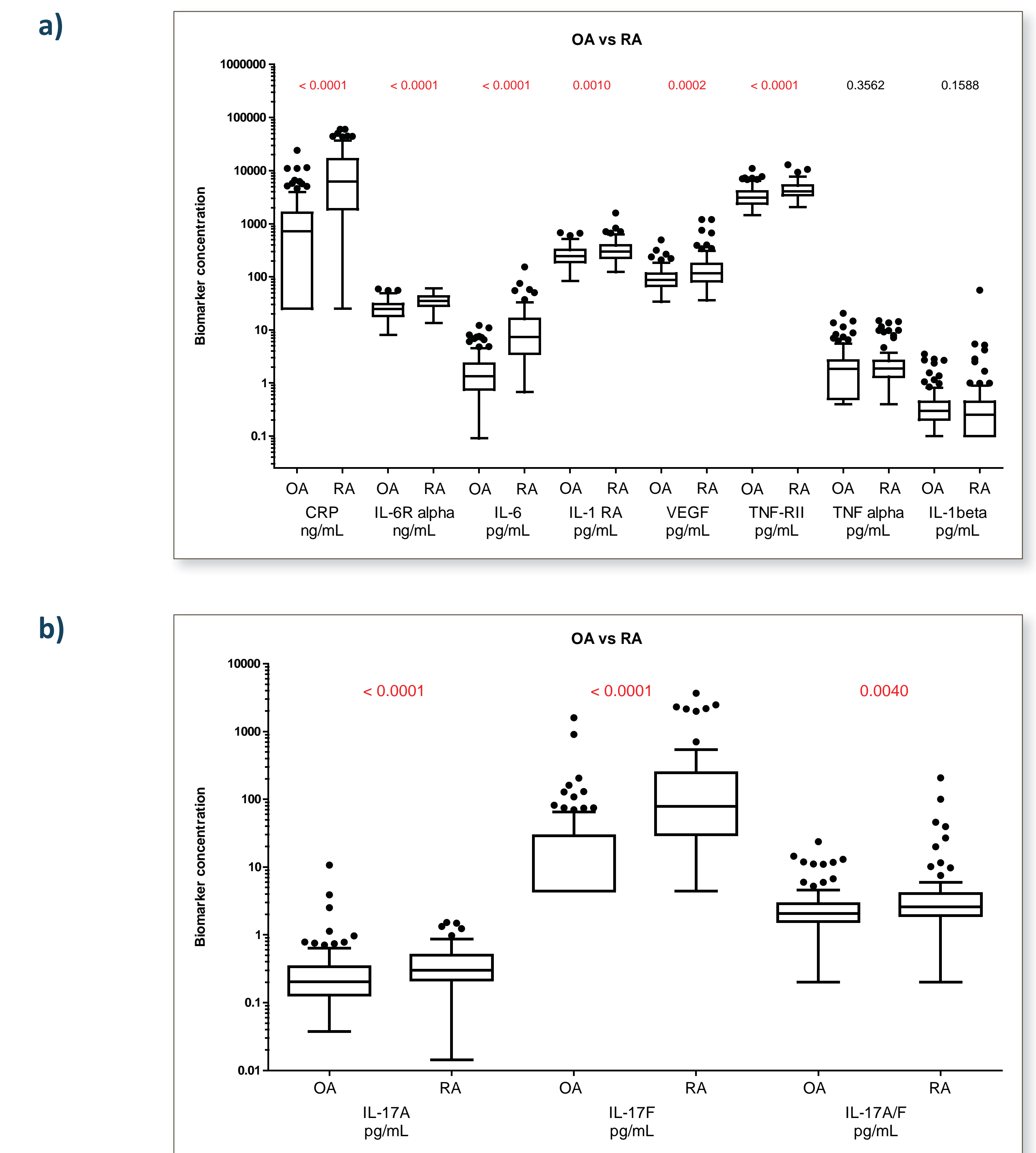


Figure 2. A panel of inflammatory biomarkers was examined in DMARD (Disease Modifying Antirheumatic Drugs)-naive RA vs knee OA (Osteoarthritis) patient cohorts (samples provided by Dr. Jeff Greenberg, NYU). **a)** Markers that exhibited significant differences in plasma concentrations between the two cohorts included CRP, IL-6R alpha, IL-6, IL-1 RA, VEGF, TNF-RII, and **b)** IL-17A, IL-17F, and IL-17A/F. Among the three tested isoforms of IL-17, the magnitude of elevation for IL-17F in RA patients was the highest. This data has been previously presented at the American College of Rheumatology 2011 by Dr. Greenberg's group.

- Singulex provides high-resolution monitoring of baseline IL-17A concentrations that are present at low levels, allowing for a clear differentiation of the disease state from the healthy population. The technology also enabled quantification of other IL-17 isoforms in RA patients, which have not been well characterized before.

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