A Cytokine/Chemokine Multiplex Assay That Is Sensitive and Specific For Fibromyalgia Compared To Controls, Systemic Lupus Erythematosus, and Rheumatoid Arthritis. Daniel J. Wallace1, Igor Gavin2, Oleksiy Karpenko2 and Bruce S. Gillis2. 1Cedars-Sinai Medical Center, Los Angeles, CA, 2University of Illinois College of Medicine atChicago, Chicago, IL.

Background/Purpose: Fibromyalgia (FM) is a chronic pain and fatigue syndrome that affects about 6 million adults in the United States. The clinical diagnosis of FM has been based on subjective evaluations paired with the exclusion of other diseases. No confirmatory objective laboratory benchmark has been available. To investigate potential FM biomarkers, we previously determined whether cytokine/chemokine production is altered in FM patients by comparing their in vitro peripheral blood mononuclear cell (PBMC) cytokine/chemokine responses to mitogenic activators with matched healthy controls. A multiplex immunoassay (Luminex™) was used on PBMC supernatants. Based on a larger multiplex array exploratory pilot study, a custom panel of antibodyconjugated beads for eight cytokines/chemokines (IL-5, IL-6, IL-8, IFN-g, IL-10, MIP-1a, MIP-1b, MCP-1) was used. This panel was 93% sensitive for the diagnosis of FM. The current study was designed to address the specificity of the panel for FM vs SLE and RA.

Methods: After IRB informed consent, a total of 25 SLE and 25 RA patients, fulfilling ACR criteria, were recruited and compared with 101 FM patients and 91 controls. Peripheral blood was harvested and PBMC isolated by differential centrifugation on Ficoll. These cells were cultured overnight in medium alone or in the presence of mitogenic activators; PHA or PMA in combination with ionomycin. The supernatant cytokine/chemokine concentrations were etermined using the Luminex multiplex immunoassay bead array technology. Data was ported to "R" statistical software for appropriate analysis. The standard curve was fitted with a 5-PL model and the concentrations of the analytes were quantified according to this curve. The two sided t-test was used to test whether the mean concentrations of each chemokine and cytokine were the same in every group. Each patient's profile was scored, as previously described, and ported to statistical software for appropriate analysis.

Results: Compared to FM, cytokine/chemokine levels from stimulated RA and SLE PBMC supernatants were significantly different (p_0.002). The concentrations of all cytokines except for IL-8 in RA were significantly lower in FM than in SLE or RA at 1% false discovery rate. In SLE and RA, 68% and 76%, respectively, tested negative for the FM profile. This was lower than healthy controls at 89%. Concomitant corticosteroid use might explain this since in steroid naïve SLE and RA, the negative FM profile increased to 86% and 83%, respectively. There was no statistically significant difference at the 95% confidence level between the concentrations of chemokines and cytokinesin SLE and RA patients except for MIP-1b (p_0.042), which was higher in the SLE cohort.

Conclusion: These data demonstrate that lowered cytokine/chemokine profiles from stimulated PBMC may represent a biomarker that is relatively sensitive and specific for FM compared to SLE and RA. It remains unclear if these differences are directly related to the pathogenesis of FM or if subsequent measurements will be useful in longitudinal outcome studies. This test may represent the first objective laboratory marker for an otherwise enigmatic and clinically defined rheumatic disease.

Disclosure: D. J. Wallace, EpicGenetics, 5; **I. Gavin**, EpicGenetics, 3; **O. Karpenko**, EpicGenetics, 3; **B. S. Gillis**, EpicGenetics, 4.