

THU0065 ASSAY DEVELOPMENT FOR PRECISE MEASUREMENT OF DISEASE ACTIVITY SERUM BIOMARKERS

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Background: Disease activity in rheumatoid arthritis (RA) is typically measured by clinical indices including the Disease Activity Score (DAS). The development of a Disease Activity (DA) test based on levels of serum protein biomarkers would provide a simple, objective and quantitative measure of biological disease activity to complement existing clinical assessment. Towards this end, we have developed a DA test incorporating panels of multiplexed immunoassays.

Objectives: Multiplexed immunoassays enable parallel testing of multiple serum biomarkers, but are susceptible to assay artifacts potentially leading to erroneous results. To ensure accuracy, assay performance must be optimized including precision, reproducibility and stability. We designed an RA Disease Activity test which eliminates these risks and provides precise measurement by understanding and optimizing design factors such as assay tolerances, RF and HAMA interference, pre-analytical biomarker stability, and diurnal variation in biomarker levels.

Methods: We characterized baseline performance for biomarker assays under consideration for a final DA test using 2 different reagent lots with 20 replicate runs each across 10 days and multiple operators. Procedural parameters were intentionally varied to determine the tolerance ranges. Interference studies were performed by spiking known amounts of the test 'interferent' into multiple subjects' sera or using sera with known levels of the 'interferent' and observing the effect on the reported biomarker levels; the effect of blocking agents was tested by adding them to this serum to assess effect. Stability studies on individual biomarkers in serum were run on 8 individual's serum samples incubated at multiple temperatures for up to 25 days as well as evaluation in multiple freeze/thaw cycles. To investigate whether the biomarkers of interest varied significantly over a 24 hour period, sera were obtained from 12 RA subjects at multiple time points over 24 hours. A final DA test algorithm was defined using a subset of the assays and the resulting DA test score was calculated for each sample.

Results: Three multiplex panels covering 17 serum biomarkers were developed and validated across 2 assay reagent lots. Baseline assay characterization intra- and inter-assay tolerances were measured: individual marker bioassay intra-assay CV's ranged from 2-8%. Tolerances for assay variables including incubation times, incubation temperatures, concentrations of key components, multiplex assay cross-reactivity, mixing, and time before reading were established. The assays are robust to routine variations in assay parameters. RF and HAMA Interference were eliminated by heterophilic blocking agents and appropriate assay diluents. The average reportable range for the multiplexed assays was 3.67 logs. Lower limits of quantification ranged from 0.32 to 4.1 x10⁵ pg/mL depending on the biomarker. The biomarkers in the final DA test are optimized for precision and reproducibility, are stable in serum for up to 3 days at room temperature, and the score derived from the biomarkers has low diurnal variation.

Conclusion: A DA test using multiplexed serum biomarker assays has been developed. Underlying assays for the biomarkers used in the DA test have been engineered to provide precise, consistent and reliable results.

Disclosure of Interest: S. Eastman Employee of: Crescendo Bioscience, W. Manning Employee of: Crescendo Bioscience, F. Qureshi Employee of: Crescendo Bioscience, D. Smith Employee of: Crescendo Bioscience, G. Cavet Employee of: Crescendo Bioscience, D. Haney Employee of: Crescendo Bioscience, Y. Shen Employee of: Crescendo Bioscience, C. Alexander Employee of: Crescendo Bioscience, L. Hesterberg Employee of: Crescendo Bioscience