CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP) ASSAY ON THE IDS-ISYS AUTOMATED SYSTEM

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Introduction

Diseases affecting the musculoskeletal system are a major cost burden to society. Articular cartilage loss or damage in these diseases is detected by radiography and measuring decreases in joint space width (JSW). The early stages of the disease may remain latent and asymptomatic for many years. In the process of joint diseases there is a progressive loss of the articular cartilage. Its matrix is degraded and proteins and/or its fragments are released into the surrounding body fluids. The increase release of proteins or its fragments to the circulation reflects an accelerated tissue turnover that can lead to permanent joint damage. This forms the rationale for applying molecular marker technology (biomarkers) to identify patients prone to rapid joint damage progression and to distinguish them from those having a better prognosis.

One such biomarker is Cartilage Oligomeric Matrix Protein (COMP, thrombospondin 5). COMP is a structural component of cartilage and stabilizes the collagen network. The molecule is a homopentamer of 435 kDa in which each individual monomer is composed of four epidermal growth factor (EGF) domains, eight thrombospondin type 3 (TSP3) domains, and a globular C-terminus. The subunits are held together by a coiled domain close to the N-terminal end, and this is stabilized by interchain disulfide bonds.

COMP can be used to monitor the progress of cartilage degradation in inflammatory joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). A great number of clinical studies have shown that measurements of serum COMP can be used a valuable tool for identifying patients at high risk from rapid joint destruction and for monitoring treatment efficacy.

Note: The IDS-iSYS COMP Assay is under development and is currently not released on the market.
The following data were produced during the feasibility and early optimization stages.

Materials and Methods

The COMP assay is based on the direct sandwich technique in which two specific monoclonal antibodies are directed against separate antigenic determinants on the COMP molecule. The capture antibody is biotinylated and binds to the solid phase of magnetic particles via the streptavidin-biotin interaction. The detector antibody is acridinium-labelled.

Standards and sera samples (diluted 1 in 20) are transferred to a cuvette, the biotinylated and acridinium-conjugated antibodies are added and incubated for 30 minutes. After incubation Streptavidin-coupled magnetic particles are added and incubated for a further 10 minutes. Following a wash step trigger solutions are added for signal detection via chemiluminescence. The amount of analyte present will be directly proportional to the Relative Light Units (RLU) quantitated by the luminometer.

Protocol
50 µL Standard/Sample
50 µL Capture Antibody
50 µL Detector Antibody

Incubate 30 minutes at 37°C

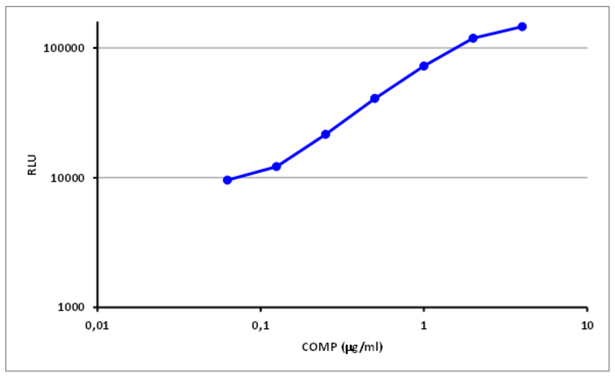
15 µL Streptavidin coated Magnetic Particles

Incubate 10 minutes at 37°C

Wash/Read

Results

Standard curve and assay range



The IDS-iSYS COMP assay has a linear range from 0.125 µg/mL to > 2 µg/mL . The standard used is human recombinant COMP diluted in equine serum-based buffer at known concentrations.

Precision

Five (5) serum samples were measured in quadruplicates at 11 time points over span of 29 days. Inter assay precision gave %CV's between 4 and 9% on dose.

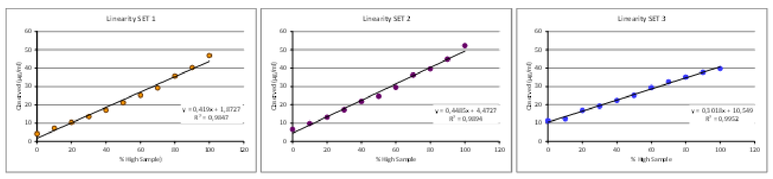
Sample ID	n	Mean (µg/ml)	SD	% CV
Sample 1	44	15,75	0,83	5
Sample 2	44	45,70	4,00	9
Sample 3	44	16,42	0,85	5
Sample 4	44	10,44	0,51	5
Sample 5	44	18,69	0,78	4

For Intra assay precision over 30 replicates the %CV was 5-8 %.

COMP (µg/ml)	n	Mean	SD	% CV
0.25	30	0,26	0,018	7
0.50	30	0,50	0,023	5
1.00	30	0,97	0,074	8

Linearity

The assay was assessed by diluting from 0 % to 100% of three high samples (spiked) with three low samples.



Linearity showed acceptable results with all the variations being within +/-10%.

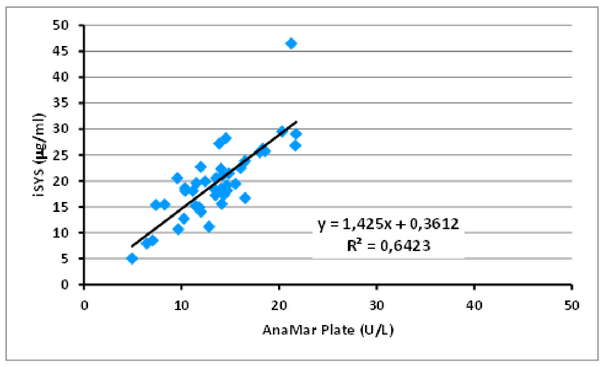
Sensitivity

The LoB was 0.011 µg/ml and the LoD was 0.027 µg/ml. The value of the LoQ from estimated concentration equivalent to 20% CV could not be determined in the samples tested; therefore the value of the lowest calibrator corresponding to 0.065 µg/ml (19.75%CV) might correspond to the LoQ. The %CV's from samples were broadly equivalent to the calibrators, however the lowest sample was 0.188 µg/ml (5.6% CV).

Summary
LoB: 0.011 µg/ml
LoD: 0.027 µg/ml
LoQ: 0.085 µg/m

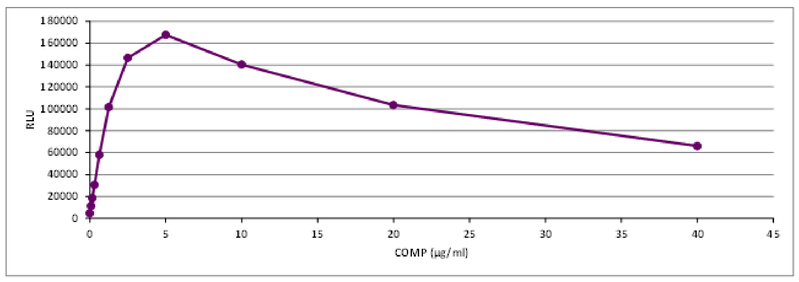
Method comparison against AnaMar COMP kit

A total of thirty-five sera samples from healthy individuals and patients with Rheumatoid Arthritis and Osteoarthritis were diluted off-board 1:20 and measured simultaneously with the AnaMar COMP kit and on the iSYS. Reported values after multiplying by the dilution factor.



Hook effect

To evaluate the Hook effect on the assay a standard curve from concentrations ranging from 40 µg/ml to 0.078 µg/ml was run in quadruplicate for each concentration tested.



The Hook effect was observed at a concentration above 5 µg/ml. A sample with a concentration up to 50 µg/ml and diluted 1:20 will give correct values.

Interfering Substances

No significant interference was observed with samples spiked with:

1. Bilirubin at 200 mg/L
2. Haemoglobin at 500 mg/dL
3. Human Serum Albumin at 12 g/dL
4. Red Blood Cells at 0.4%
5. Triglycerides at 3000 mg/dL

Conclusion

The IDS-iSYS COMP prototype assay developed at the University of Lund shows good performance for the items tested including precision, linearity, sensitivity, hook effect and interfering substances.