

Development of a Hyaluronic Acid ELISA for Clinical Use

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Introduction

Hyaluronic acid (HA), hyaluronate or hyaluronan is a non-sulfated glycosaminoglycan. As a major component of connective tissues it is distributed ubiquitously in the organism. Serum HA is a sensitive marker in clinical diagnosis, also it is used to monitor disease progression as well as to judge the success of appropriate therapies of patients with degenerative joint disease, as well as risk of progressive liver fibrosis and cirrhosis.

Joint disease

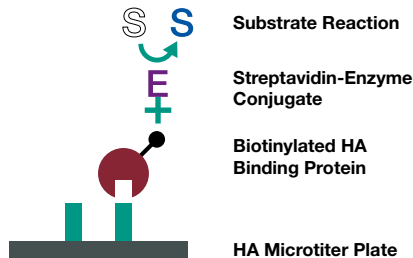
Proliferative synovial inflammation – which is a key feature of rheumatoid arthritis (RA) – causes the forced synthesis of HA increasing both the synovial and serological level of HA. However, joint inflammation might also occur during other types of joint diseases, e.g. osteoarthritis (OA) or traumatic injury. In RA and OA patients the concentration of HA correlates with the degree of joint inflammation and synovial proliferation as well as with the degree of joint space narrowing.

Liver disease

As a result of permanent inflammation most chronic liver diseases are characterised by fibrosis and cirrhosis causing a decreased capacity for HA clearance. The leading causes of chronic liver disease are viral infection (hepatitis B or C), and alcohol abuse. Patients with extensive liver fibrosis and cirrhosis show markedly increased serum levels of HA with the progression of liver fibrosis being associated with an increase of serum HA.

Methods

Test Principle

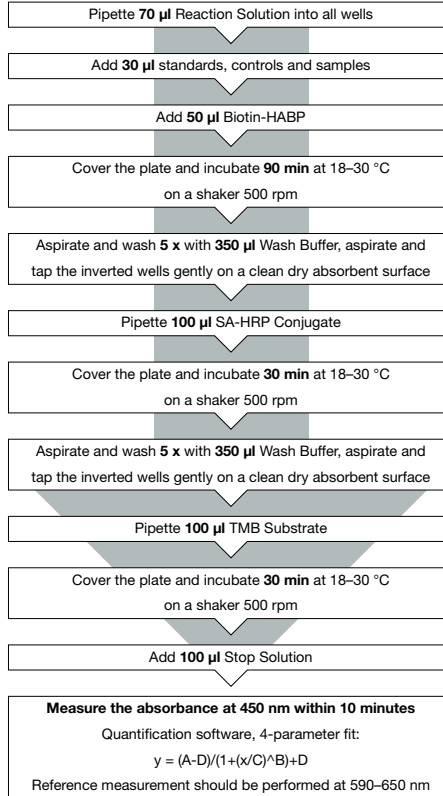


The test is using a HA-specific biotinylated binding protein and HA microtiter plates. This biotinylated binding protein binds to immobilized HA, if not saturated by free HA of the samples, followed by a streptavidin-peroxidase conjugate and a substrate reaction. 30 µl of undiluted specimens can be assayed within 2.5 hours.

Sample collection

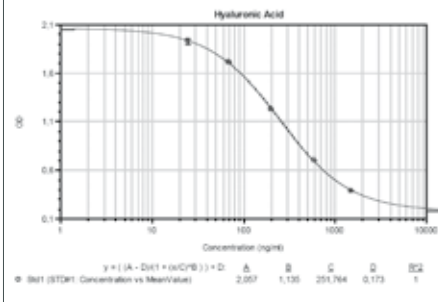
Fasting blood collection. Hyaluronic Acid Values are dependent on age and sex and influenced by food intake and physical activity. Values in EDTA-Plasma are 10 % lower.

Assay Procedure



Results

Standard Curve



Test Characteristics

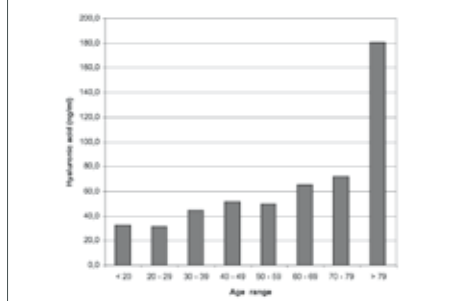
Standard range	25–1500 ng/ml		
Sensitivity	13.3 ng/ml		
Intra-assay CV	Mean (ng/ml)	SD (ng/ml)	CV (%)
Sample 1	104	7.1	6.8
Sample 2	523	31.3	6.0
Inter-assay CV	Mean (%)	SD (%)	CV (%)
Sample 3	65	5.1	7.8
Sample 4	378	22.5	6.0
Parallelism	99.2	6.9	
Recovery	104.4	6.4	

Results

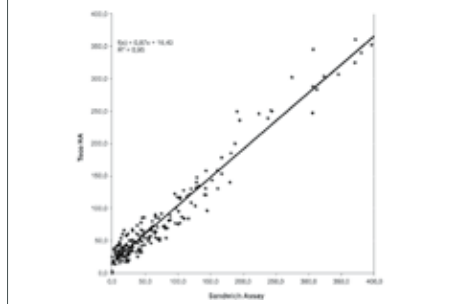
Values in Normal Subjects

	n	Mean (ng/ml)	Range (ng/ml)	(Mean \pm 2*SD)
Female premenopausal	20	31.8	13.9	49.7
postmenopausal	16	58.4	33.3	83.5
Male	17	50.0	20.3	79.6
all Subjects < 80 years	53	45.6	12.7	78.5
Cut-off:			80 ng/ml	

Values Normal Subjects Age Dependend



Correlation



Conclusion

With a measuring range of 25–1500 ng/ml, the analytical sensitivity was at 13.3 ng/ml. The mean recovery of hyaluronic acid in serially diluted human serum samples with medium or high internal HA levels was 99 % of the expected values. The mean recovery of HA added to different human sera was 104 % of the expected values. Intra-assay coefficient of variation was below 7 %, the inter-assay coefficient below 8 %. Despite plasma samples were 10 % lower, their correlation to the corresponding serum samples was 99 %. For the evaluation of the assay, serum samples from 53 healthy individuals (16 to 79 years, mean 45.5 years) were tested. The mean hyaluronic acid concentration was 45.6 \pm 16.5 ng/ml. Based on these values a cut-off of 80 ng/ml (mean + 2*SD) has been defined. Serum hyaluronan is influenced by various factors including age. In the group of 20–30 year old subjects we found HA-levels of 31.5 \pm 9.5 ng/ml, in contrast to 72.0 \pm 36.7 ng/ml in 70–80 year old individuals. In summary, the developed ELISA was optimized, standardized and tested for routine use so that it is a sensitive and reliable method for measuring hyaluronic acid in human plasma, serum and other biological fluids.