MEASUREMENT OF HA LT

The latex agglutination method by Wako (HA LT) shows excellent analytical characteristics making it a superior tool for clinical practice. Total automation on various biochemical clinical analysers is possible. Using an Olympus AU640 analyzer Guéchot et al. reports the HA LT precision for within-run CV to be ≤ 2,12 % and for between-run CV to be ≤ 4,08 % (Tab 2).

Table 2: Within-run and between-day standard deviations using HA LT [15]

	Pool A (µg/L)	Pool B (µg/L)	Pool C (µg/L)
Within-run imprecision (20 determinations in the same run)			
Mean	48.7	174.4	901.8
S.D.	0.92	3.69	8.48
CV	1.90 %	2.12 %	0.94 %
Between-run imprecision (1 determination per day for 20 days)			
Mean	49.9	171.7	900.7
S.D.	2.03	4.15	24.66
CV	4.08 %	2.42 %	2.74 %

The analytical sensitivity was determined to be 1,5 ng/mL with 99 % confidence and the standard curve showed linearity up to 1000 ng/mL. Serum and heparinised plasma can equally be used. Correlation studies between HA LT and ELISA assay using 138 clinical samples show no inaccuracies or significant differences between the methods (Fig 2) [15]. Another study reported excellent correlation of HA results on different analysers and labs [16].

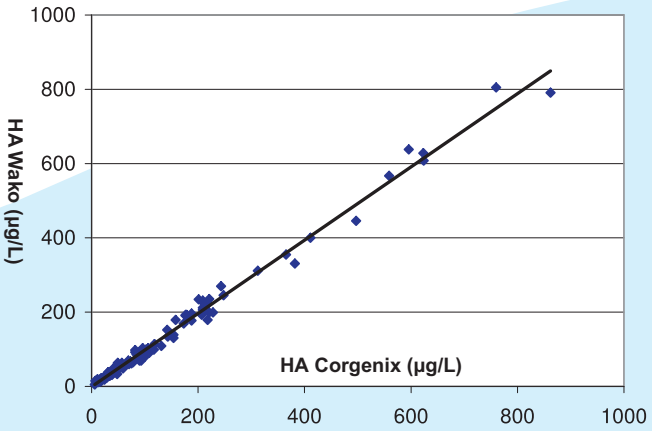

Fig. 2: Comparison between HA LT by Wako and HA ELISA

Rev. 0813K3

ORDERING

HA LT can be ordered through Wako Chemicals GmbH (see contact Info below).

Code No.	Product	Package
992-71185	Hyaluronic acid LT	R1: 2 x 15 mL, R2: 2 x 6 mL
993-71095	Hyaluronic acid LT	R1: 2 x 31 mL, R2: 2 x 11 mL
993-71115	HA Calibrator Set	CAL: 5 conc. X 2 mL
998-71165	HA Control Set	CTR: 2 x 2 conc. X 2 mL

APPLICATIONS (SELECTION)

Manufacturer	Instrument type
Abbott/Toshiba	C8000 Architect/TBA-120
Beckman Coulter/Olympus	AU400, AU600, AU640, AU2700, AU5400
Roche/Hitachi	Hitachi 912, Hitachi 917, Modular
Roche	Cobas 6000
Siemens/Jeol	Advia 1800/BM 6050, BM 1650

Names of companies and instruments are trademark protected.

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Wako Chemicals GmbH

Wako

- Liver fibrosis
- Diagnostics
- Significance

HA LT

LIVER FIBROSIS

Liver fibrosis is the result of chronic injury. There are different aetiologies, prominently viral hepatitis, alcoholism and fatty liver disease. Worldwide about 400 million people are chronically infected with hepatitis B and about 130 million with hepatitis C virus. In Europe alcoholic and non-alcoholic liver diseases (ALD/NALD) are also prevalent factors leading to liver fibrosis. This injury results in expansion of extracellular matrix (ECM) and distortion of normal architecture of the liver and plays a direct role in hepatocellular dysfunction and hypertension. Fibrosis can progress to cirrhosis with life-threatening complications like hepatocellular carcinoma (HCC).

DIAGNOSTICS

An accurate determination of the extent of fibrosis is essential for planning a specific antifibrotic treatment. In the follow-up of treated patients regular diagnostics is important to monitor the success of treatment.

Today percutaneous liver biopsy is considered to be the gold standard for diagnosing and staging of liver disease. However biopsy shows complications including pain (10-30% of the cases), bleeding (resulting in a prolonged hospital stay) and finally mortality (0,01 – 0,1 %). Among its technical limitations, sampling errors play an important role. Only 1/50.000 of the liver is sampled, but fibrosis may not be equally distributed throughout this organ. The subjective interpretation of biopsy samples furthermore can lead to interobserver variation [1]. Another shortcoming of liver biopsy is its cost as it typically requires hospitalization.

To overcome these limitations non-invasive procedures such as elastography and biochemical markers have been proposed [1]. Among the biochemical markers hyaluronic acid (HA) is described by the literature to be the most valuable single biomarker in discriminating advanced from no fibrosis.

WHAT IS HYALURONIC ACID?

Hyaluronic acid (HA) is a high molecular weight glycosaminoglycan. Repeating units of a disaccharide composed of β-(1-4)-glucuronic acid and β-(1-3)-N-acetylglucosamine form a linear polysaccharide that varies in length from 10 to more than 1000 units. HA is produced by fibroblasts throughout the body and plays a structural role in the connective tissue matrix.

While high concentrations of HA can be found in different connective tissues and joints, serum levels of HA in healthy individuals are typically low due to effective degradation by the sinusoidal endothelial cells (SEC) of the liver. In various liver diseases, particularly in cirrhosis, serum HA concentrations increase significantly mostly due to the reduction in HA receptors of SECs and the amount of HA binding to the cells [2]. Thus a correlation between serum HA levels and histopathological changes in the liver has been described by many investigations during the last two decades.

WHAT IS HA LT ?

The CE-marked Hyaluronic acid Latex Agglutination method by Wako (HA LT) is a fully automated assay to determine HA concentrations in serum and plasma. It can be performed by common biochemical analysers. The patient sample is mixed with a recombinant hyaluronic acid binding protein (HABP). Latex particles coated by anti-HABP antibodies are added and bind to HABP - HA complexes resulting in increasing turbidity. The degree of turbidity is proportional to the concentration of HA in the sample (Fig. 1).

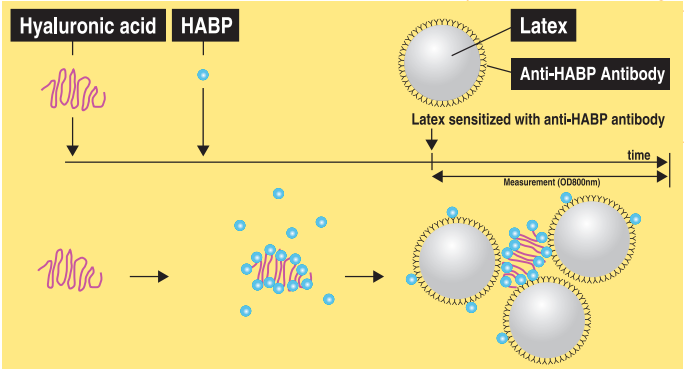

Fig. 1: Principle of the HA LT assay

CLINICAL SIGNIFICANCE OF HA

HA has been extensively studied in patients with different aetiologies of liver diseases. It proved to be the best single biomarker of fibrosis with high sensitivities and specificities to discriminate cirrhosis from other fibrosis stages. Using a cut-off concentration of 60 ng/ml HA shows typically a negative predictive value (NPV) of up to 100 % and a positive predictive value (PPV) of 61 % for cirrhosis [3], which indicates the unique diagnostic power of this marker to exclude cirrhosis by a non-invasive procedure. Its diagnostic accuracy outperforms that of another prominent marker, procollagen-III-peptide (PIIIP), both for discriminating HCV-patients with extensive fibrosis from those with no or mild fibrosis, and for discriminating patients with cirrhosis from those without cirrhosis [4]. Table 1 summarises important results regarding the diagnostic performance of HA in patients with different aetiologies of liver diseases.

Table 1: Diagnostic performance of HA in patients with different aetiologies

Aetiology	Diagnostic performance of HA	Reference
Hepatitis C Virus (HCV)	100 % NPV for cirrhosis (cutoff 50 ng/mL)	Halfon P et al. [5]
Hepatitis B Virus (HBV)	90,9 % sensitivity, 98,1 % specificity for extensive fibrosis (cutoff 126,4 ng/mL)	Montazeri G et al. [6]
Alcoholic Liver Diseases (ALD)	82,8 % sensitivity, 69 % specificity for hepatic fibrosis, > Ludwig stage 2 (cutoff 55,5 ng/mL); continuous rising HA concentration during progress of liver damage	Stickel F et al. [7]
Non-Alcoholic Fatty Liver Disease (NAFLD)	85 % sensitivity, 80 % specificity for severe fibrosis (cutoff 46,1 ng/mL)	Suzuki A et al. [8]
C282Y Hemochromatosis (HH)	100 % sensitivity and specificity for cirrhosis (cutoff 46,5 ng/mL) in patients with ferritin > 1000 ng/mL	Crawford DHG et al. [9]
Thalassemia	Elevated HA levels in patients with chronic HCV, but no correlation to ferritin or liver iron content; role as fibrosis marker suggested	Papastamataki M et al. [10]

Several combinations of biomarkers in indices or panels have been established. The values of at least two markers are typically used to calculate scores using algorithms and show improved diagnostic accuracy for individual stages of hepatic fibrosis. Several of these prominent scores include HA.

Fibrometer includes HA and has proven to be a superior tool for pathological staging and for the estimation of the area of liver fibrosis [11].

Hepascore also includes HA and three other biochemical tests in addition to age and sex. Investigators have reported high diagnostic performance for significant fibrosis and cirrhosis among HCV patients with the use of HA [12]. Using HA LT, Hepascore® can be made fully automated and can be implemented in routine clinical practice [13]. A multicentre, prospective study in 19 French medical centres underlines the role of Hepascore. It showed similar diagnostic performance compared to Fibrotest®, Fibrometer® and Fibroscan™. However in 22 % of the cases Fibroscan™ was not interpretable [14].

The use of HA LT can significantly improve such scores in terms of automation, simplicity and analytical performance.