Hyaluronic acid LT

Read this instruction sheet thoroughly before use.

Latex Agglutination Method

For the quantitative determination of Hyaluronic Acid (HA) in Serum and Plasma

992-71185
0613 D1

Intended use

Hyaluronic acid LT is an *in vitro* assay for the quantitative determination of hyaluronic acid (HA) in serum and plasma.

Summary and explanation of the test

Hyaluronic acid is an unbranched glycosaminoglycan, a single chain of polymers of disaccharide units containing N-acetylhexosamine and hexose.[1] Hyaluronic acid is widely distributed in connective tissue and produced mainly in mesenchymal cells.[2] Hyaluronic acid has several functions, e.g. lubrication in joints, prevention from bacterial invasion and internal body hydration.

Hyaluronic acid is the best marker to date for serially assessing liver cirrhosis. Serum concentration of HA is consistent with stage of fibrosis, and also decreases with a response to interferon therapy in patients with HCV chronic infection.[3-9]

Hyaluronic acid LT is a test kit for the quantitative determination of hyaluronic acid based on the latex agglutination method, and this method can be applied to general clinical chemistry analyzers.

Principle of the method

A sample is mixed with a hyaluronic acid binding protein (HABP), and hyaluronic acid in the sample combines specifically with HABP. In order to make an insoluble aggregate, latex particles coated by anti-HABP antibody are added, and the latex binds to above complex. As a result, the insoluble aggregate increases turbidity in the solution. The degree of turbidity of solution can be measured optically and is proportional to the concentration of hyaluronic acid in the sample.

Reagents

Contents and storage conditions

R1:	HABP Reagent	Store at 2 - 10°C*
R2:	HA Latex Reagent	Store at 2 - 10°C* (*Do not freeze)
Ingredients		
R1: **HABP Reagent**	Recombinant hyaluronic acid binding protein (rHABP)	
	Bovine Serum Albumin	0.1%
	Sodium Azide	0.09%
R2: **HA Latex Reagent**	Latex sensitized with anti-HABP antibody (mouse, monoclonal)	
	Boric Acid	0.31%
	Bovine Serum Albumin	0.5%
	Sodium Azide	0.09%

Reagent preparation

R1: Use HABP Reagent as supplied. After opening bottle, store at 2 - 10°C and use within one month.
R2: Use HA Latex Reagent as supplied. After opening bottle, store at 2 - 10°C and use within one month.

Specimen collection and preparation

Use serum or plasma as a specimen.
Assay samples immediately after collection.
Stability of HA in the sample is summarized below. All results come from in-house evaluation. Stability of HA in the sample should be different due to sample characteristics.
It is recommended that specimen collection is carried out in accordance with local and national regulations. Since all specimens are potentially infectious, they should be handled in accordance with any other local or national regulation relating to the safe handling of such materials.

Storage temperature	Stability of HA in the sample
-80°C	Stable up to 26 months.
-30°C	Stable up to 26 months.
7°C	About 7% decrease after 60 days.
26°C	About 11% decrease after 14 days.

Physical or chemical indications of instability

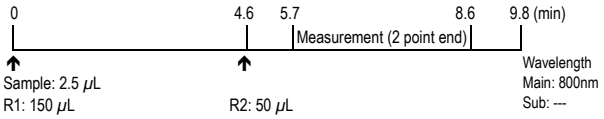
The presence of precipitates in the reagents or values of control materials outside the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

The reagent is designed to be used on commercially available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications. A validation by the user in practice at the customer's site in the form of measurements of adequate control or patient sera in sufficient number is indispensable.

Standard procedure

Temperature: 37°C (Hitachi®917s)



Calibrator: HA Calibrator Set (Available separately).

Application to the various automatic analyzers

Input the parameters according to the instructions of instruments to perform the measurement. Instrument applications are available upon request.

Calibration

The calibration curve is automatically produced in the automated analyzer by plotting absorbance (turbidity) vs. concentration. Use saline for blank. Refer to the operator's manual for details on performing calibration. Consult the instrument manufacturer for details.

Results

The final results are automatically calculated and printed in concentration. The results are given in ng/mL.

Expected values[10]

The expected value for hyaluronic acid has been reported to be 23 ± 17 ng/mL.

Limitations of the procedure

(1) When the nonspecific reactant (e.g. heterophilic antibody) exists in a specimen, the correct measurement result may not be obtained. The results should be used in conjunction with physician judgment and symptoms.

(2) The linearity of Hyaluronic acid LT is up to 1000 ng/mL. If hyaluronic acid value exceeds the upper limit of measurable range, dilute the sample with saline, repeat the assay and multiply the result by the dilution factor. When the concentration of hyaluronic acid in the sample is 1000 - 50000 ng/mL, measured value does not fall below 1000 ng/mL.

Warnings and precautions

- For *in vitro* diagnostic use.
- The usage and application of this test is reserved for professional use only. Please refer to respective national and local regulations and legislation.
- Not to be used internally in humans and animals.
- Do not mix reagents, which have different lot numbers.
- Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on the bottle.
- Do not use the reagents described above for any purpose other than described herein.
- Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures.
- Operate the instruments according to operator's manuals under appropriate conditions. Consult the instrument manufacture for details.
- Do not use reagent that was frozen by mistake.
- After opening reagents, it is recommended to use them immediately. When the opened reagents are stored, cap the bottles and keep them under the specified conditions.
- Do not use the containers or other materials in the kit for any purpose other than those described herein.
- **Before use of HA Latex Reagent, homogenize the reagent by slowly turning the bottle upside down.**
- A calibration material is sold separately. For the usage of calibration material, refer to its package insert.
- In some instances, falsely high or low result occurs due to non-specific turbidity. If a result is questionable, inspect the reaction course or dilute the sample and repeat analysis.
- Intake of food results in elevation of serum or plasma hyaluronic acid concentration. Use fasting serum or plasma as a specimen.[11]
- If the reagents come in contact with mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- All materials and apparatus that may be contaminated with specimen should be treated with caution to avoid infection.
- All devices including reagents and reagent bottles that come in contact with specimen should be considered potentially infectious.
- R1 and R2 Reagent contain 0.09% sodium azide as a stabilizer. Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though this product contains minute quantities of sodium azide, drains should be flushed well with a large amount of water when discarding the reagents.
- Reagents contain 3090 mg/L boric acid (540 mg/L as boron).
- Wear protective lab gear when disposing of the liquid waste, sample cup and measurement disk to avoid infection.
- When discarding the reagents, dispose of them according to local or national regulations.

Interfering substances

a) Ascorbic acid, bilirubin and hemolysis do not have significant effects on the assay.

b) Anticoagulants such as heparin, citrate, oxalate and EDTA, and sodium fluoride as a glycolytic inhibitor, do not have significant influences on the assay when they are used in their usual amounts.

Performance characteristics

Performance data were obtained using Hitachi®917s.

Linearity

Hyaluronic acid concentrations up to 1000 ng/mL were linear in the evaluation performed according to CLSI protocol EP6-A.

Sensitivity

The limit of detection of this method is 5.8 ng/mL. The limit was determined according to CLSI protocol EP17-A.

Manufactured by:

Wako Chemicals GmbH
Fuggerstraße 12, D-41468 Neuss
Telephon(e): +49-2131-311-0
Facsimile: +49-2131-311-100
www.wako-chemicals.de

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Precision

[Within-run precision]

Below are representative data of the within-run precision. The CV of hyaluronic acid obtained by measuring control sera 21 times was not more than 4.3% at 38.7 ng/mL or higher hyaluronic acid concentrations.

Sample No.	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	38.7	307.9	914.4
SD (ng/mL)	1.7	2.8	8.1
CV (%)	4.3	0.9	0.9

[Total precision]

Below are representative data of the total precision. All data were collected in accordance with CLSI protocol EP5-A.

Sample No.	Sample 1	Sample 2	Sample 3
Total mean (ng/mL)	38.5	308.7	909.2
Total precision ST (ng/mL)	2.04	7.39	18.3
Total precision CV (%)	5.3	2.4	2.0

Specificity

Specificity was studied by adding substances that were thought to endogenously interfere with hyaluronic acid test result, into serum.

Hemoglobin	(mg/dL)	None	100	200	300	400	500
Hyaluronic acid	(ng/mL)	67.0	66.4	68.8	63.2	66.7	62.8
Recovery	(%)	100.0	99.1	102.7	94.3	99.6	93.7

Bilirubin	(mg/dL)	None	10	20	30	40	50
Hyaluronic acid	(ng/mL)	68.2	65.9	65.5	64.7	65.0	66.2
Recovery	(%)	100.0	96.6	96.0	94.9	95.3	97.1

Conjugated bilirubin	(mg/dL)	None	10	20	30	40	50
Hyaluronic acid	(ng/mL)	69.4	64.3	67.2	66.8	67.1	65.8
Recovery	(%)	100.0	92.7	96.8	96.3	96.7	94.8

Intrafat	(%)	None	1.0	2.0	3.0	4.0	5.0
Hyaluronic acid	(ng/mL)	68.0	68.4	69.5	69.4	65.5	67.5
Recovery	(%)	100.0	100.6	102.2	102.1	96.3	99.3

Ascorbic acid	(mg/dL)	None	10	20	30	40	50
Hyaluronic acid	(ng/mL)	62.1	66.2	66.3	63.9	63.2	62.5
Recovery	(%)	100.0	106.6	106.8	102.9	101.8	100.6

EDTA-2Na	(%)	None	0.1	0.2	0.3	0.4	0.5
Hyaluronic acid	(ng/mL)	65.0	66.2	63.5	65.8	65.6	68.3
Recovery	(%)	100.0	101.8	97.7	101.2	100.9	105.1

Sodium citrate	(%)	None	0.4	0.8	1.2	1.6	2.0
Hyaluronic acid	(ng/mL)	65.1	61.5	65.5	62.8	65.8	67.4
Recovery	(%)	100.0	94.5	100.6	96.5	101.1	103.5

Ammonium oxalate	(%)	None	0.2	0.4	0.6	0.8	1.0
Hyaluronic acid	(ng/mL)	74.6	76.3	76.4	73.9	74.0	77.6
Recovery	(%)	100.0	102.3	102.4	99.1	99.2	104.0

Sodium fluoride	(%)	None	0.6	1.2	1.8	2.4	3.0
Hyaluronic acid	(ng/mL)	76.4	75.2	74.7	72.3	74.4	74.3
Recovery	(%)	100.0	98.4	97.8	94.6	97.4	97.3

Heparin sodium	(%)	None	0.02	0.04	0.06	0.08	0.10
Hyaluronic acid	(ng/mL)	99.4	101.8	101.7	101.9	102.8	103.5
Recovery	(%)	100.0	102.4	102.3	102.5	103.4	104.1

Accuracy

The accuracy of this method was determined by a recovery study.

Serum specimen 1

Added	(ng/mL)	0.00	40.0	100.0	200.0
Measured		36.5	83.7	140.8	235.6
		39.2	80.1	141.3	242.9
		40.2	79.9	142.1	240.9
Mean	(ng/mL)	38.6	81.2	141.4	239.8
Obtained	(ng/mL)	---	42.6	102.8	201.2
Recovery	%	---	106.5	102.8	100.6

Serum specimen 2

Added	(ng/mL)	0.00	40.0	100.0	200.0
Measured		316.5	354.9	415.8	513.9
		313.2	355.3	419.6	519.4
		308.9	357.1	419.0	510.9
Mean	(ng/mL)	312.9	355.8	418.1	514.7
Obtained	(ng/mL)	---	42.9	105.2	201.8
Recovery	%	---	107.3	105.2	100.9

Serum specimen 3

Added	(ng/mL)	0.00	40.0	100.0	200.0
Measured		751.6	794.8	868.1	943.3
		757.1	796.6	865.5	972.0
		752.5	789.2	857.0	968.5
Mean	(ng/mL)	753.7	793.5	863.5	961.3
Obtained	(ng/mL)	---	39.8	109.8	207.6
Recovery	%	---	99.5	109.8	103.8

The recovery rate of hyaluronic acid is 99.5% - 109.8% in the concentration ranges shown in the above table.

Correlation

A comparison of Hyaluronic acid LT and ELISA (a product of company A) was performed using a Hitachi®917s. The test results provided the following data:

Specimen	Serum
Correlation coefficient	r = 0.995 (n = 133)
Regression equation	y = 1.004x + 1.917
x	A product of company A (ng/mL, serum)
y	Hyaluronic acid LT (ng/mL, serum)

x (ng/mL)	Min = 5, Max = 805, Mean = 113
y (ng/mL)	Min = 6, Max = 862, Mean = 115

A comparison of serum and plasma sample with Hyaluronic acid LT was performed using a Hitachi®917s. The test results provided the following data:

Correlation coefficient	r = 0.998 (n=36)
Regression equation	y =1.013x + 0.275
x	Serum (ng/mL)
y	Plasma (ng/mL)

x (ng/mL)	Min = 7, Max = 221, Mean = 63
y (ng/mL)	Min = 10, Max = 219, Mean = 64

Quality control

A quality control program is recommended for all clinical laboratories. The analysis using Wako's HA Control Set with each assay is recommended for monitoring the performance of the procedure. The values obtained for the controls should fall within ± 20% from the assigned values.

References

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Ordering information

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	CONTROL: 2 x 2 conc. x 2 mL

Manufactured by:

Wako Chemicals GmbH
Fuggerstraße 12, D-41468 Neuss
Telephon(e): +49-2131-311-0
Facsimile: +49-2131-311-100
www.wako-chemicals.de