

Development of a hyaluronic acid detection reagent using latex agglutination method

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Introduction

Hyaluronic acid (HA) is an unbranched glycosaminoglycan, a single chain of polymers of disaccharide units containing N-acetylhexosamine and hexose (Fig. 1). Its molecular weight usually reaches millions ranging from 2-6 X 10⁶. HA is widely distributed in connective tissue and produced mainly in mesenchymal cells. HA 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. A new HA detection reagent was developed using the latex agglutination method since this method can be applied to general clinical chemistry analyzers.

Method and Procedure

An assay principle was summarized in Figure 2. A sample is mixed with a hyaluronic acid binding protein (HABP), and HA 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 HA in the patient's serum. The performance of the HA detection reagent was evaluated by using general clinical chemistry analyzer, and the standard assay procedure was shown in Figure 3.

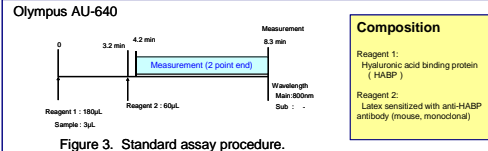


Figure 3. Standard assay procedure.

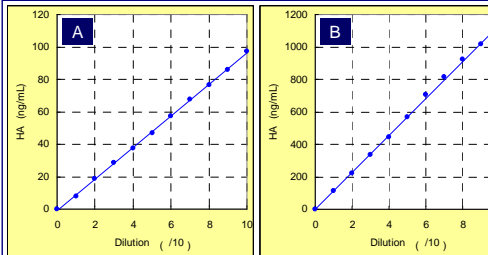


Figure 4. Linearity. (A): Low HA concentration, (B): High HA concentration

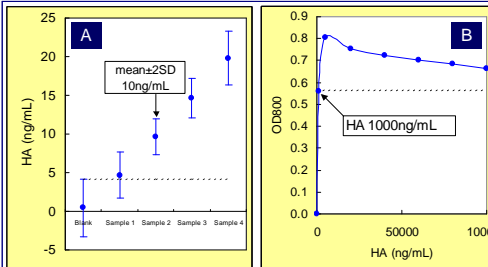


Figure 5. (A): The minimum detection limit and (B): Prozone.

Table 1. Within run-precision.

	Sample 1	Sample 2	Sample 3
n	21	21	21
Average	39.8 ng/mL	314.8 ng/mL	944.8 ng/mL
Range	6.0 ng/mL	17.0 ng/mL	42.7 ng/mL
SD	1.40 ng/mL	5.06 ng/mL	12.51 ng/mL
CV	3.5 %	1.6 %	1.3 %

Table 2. Total precision.

	Sample 1	Sample 2	Sample 3
n	21	21	21
Average	40.6 ng/mL	323.9 ng/mL	970.0 ng/mL
Range	6.2 ng/mL	18.8 ng/mL	55.1 ng/mL
SD	1.73 ng/mL	5.52 ng/mL	14.40 ng/mL
CV	4.3 %	1.7 %	1.5 %

The experiment was conducted according to the method of EP5-A of the NCCLS guideline.

Table 3. Accuracy.

	Sample 1			
Added	0 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL
Average (n=3)	36.4 ng/mL	85.7 ng/mL	133.6 ng/mL	277.6 ng/mL
Obtained	49.3 ng/mL	97.2 ng/mL	241.2 ng/mL	
Recovery	-	99%	97%	96%

	Sample 2			
Added	0 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL
Average (n=3)	357.8 ng/mL	405.6 ng/mL	460.9 ng/mL	615.4 ng/mL
Obtained	47.8 ng/mL	103.1 ng/mL	257.6 ng/mL	
Recovery	-	96%	103%	103%

	Sample 3			
Added	0 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL
Average (n=3)	748.3 ng/mL	792.7 ng/mL	864.6 ng/mL	986.7 ng/mL
Obtained	44.4 ng/mL	116.3 ng/mL	238.4 ng/mL	
Recovery	-	89%	116%	95%

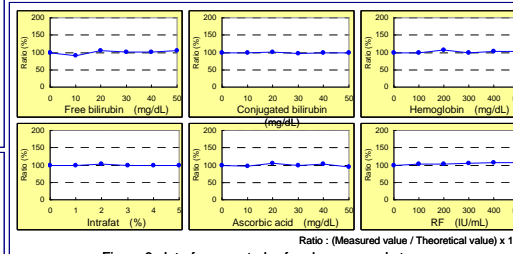


Figure 6. Interference study of endogenous substances.

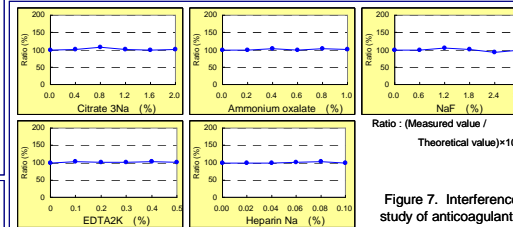


Figure 7. Interference study of anticoagulants.

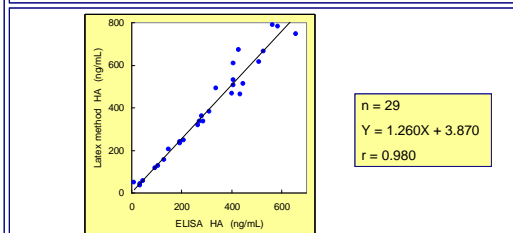


Figure 8. Correlation between latex method and ELISA.

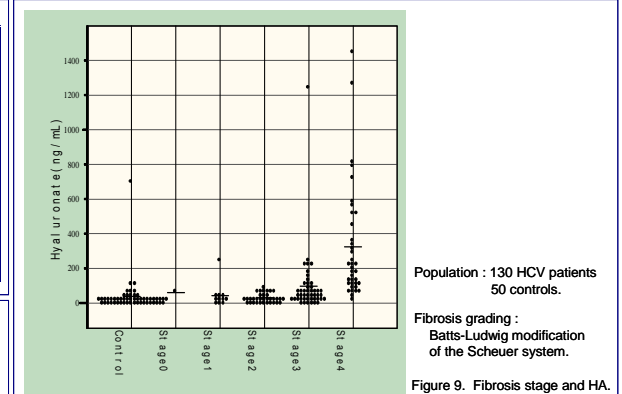


Figure 9. Fibrosis stage and HA.

Results

- Linear dose-response relationship was obtained up to 1000 ng/mL (Fig. 4).
- The minimum detection limit of HA was 10 ng/mL, and prozone phenomenon is not observed up to 100,000 ng/mL (Fig. 5).
- Within run-precision and total precision were examined by analyzing 3 serum samples (low, middle and high HA conc.). The CVs in within run were 1.3-3.5%, and the CVs in total precision were 1.5-4.3% (Table 1).
- The recovery of HA was 89-116% (Table 3).
- Several endogenous substances including hemoglobin, free-bilirubin, conjugated bilirubin, ascorbic acid, rheumatoid factor, and intrafat were found to have negligible effect (< 7%) on the assay results (Fig. 6).
- Anticoagulants such as EDTA2Na, ammonium oxalate, sodium citrate, sodium Fluoride, and heparin sodium did not interfere with the measurement results of assay (< 8%) (Fig. 7).
- The results of the present method were compared with commercially available enzyme-linked immunosorbent assay (ELISA) method using 29 patients with HCV-related chronic hepatitis. The coefficient of correlation (r) was 0.980 and the regression formula was $y = 1.260x + 3.870$ (Fig. 8).
- Relationship between HA concentration in the present method and fibrosis grading (Batts-Ludwig modification of the Scheuer system) was evaluated using 130 patients with HCV-related chronic hepatitis and 50 healthy volunteers as control. As a result, HA concentration in HCV patients with stage 3 and 4 showed significantly higher HA levels compared to controls ($p=0.0066$ and <0.0001 , respectively) (Fig. 9).

Conclusion

The present method was demonstrated to be precise, have a wide linear measurement range, and be applicable to general clinical chemistry analyzer. In addition, the present HA assay was relatively free of interference effects from elevated hemoglobin, bilirubin, ascorbic acid, intrafat and anticoagulants. This HA test showed a good ability to detect stages 3 and 4 fibrosis in chronic hepatitis patients. Therefore, the developed HA assay can be used in clinical laboratories to evaluate liver fibrosis.

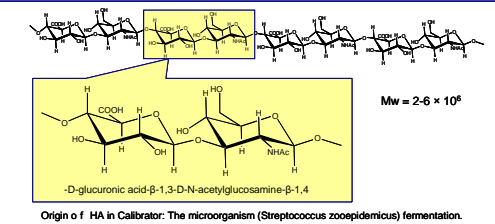


Figure 1. Structure of hyaluronic acid.

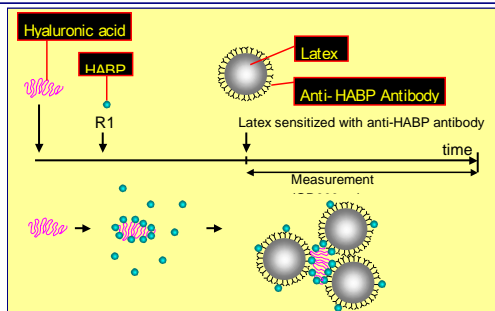


Figure 2. Assay principle.