Evaluation of an automated hyaluronan latex agglutination assay enables the implementation of routine laboratory HEPASCORE calculation

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Introduction

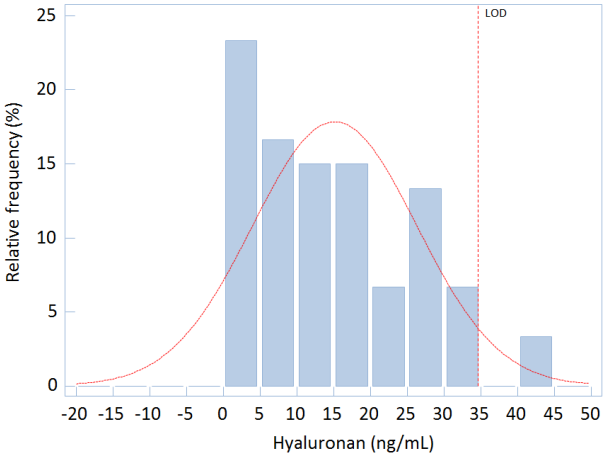
The non-invasive HEPASCORE was reported as a diagnostic tool for predicting liver fibrosis. To date, hyaluronan (HA) is still predominantly assayed by time-consuming and costly enzyme-linked protein binding assay (ELISA).

Methods

We aimed to evaluate the analytical performance of a novel automated HA-latex agglutination assay (WAKO, Osaka, Japan) using KoneLab 30i analyzer (ThermoFisher Scientific, Hudson, New Hampshire, USA) and compared HA-concentrations in healthy blood donors (N=57) to the reference HEPASCORE-ELISA (HA Test Kit, Corgenix, Westminster, USA).

Results

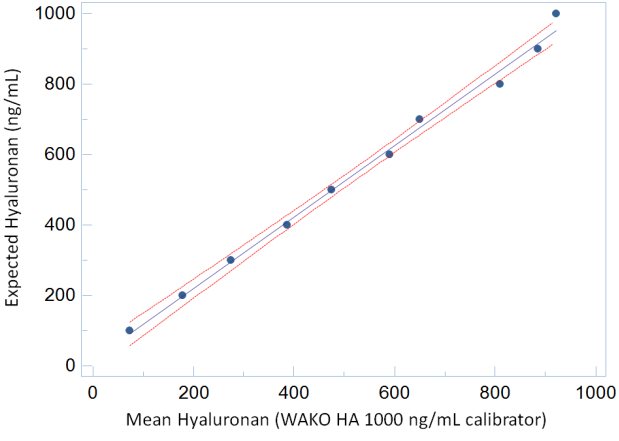
The lowest detectable level of automated HA was found to be 34 ng/mL (95. percentile).

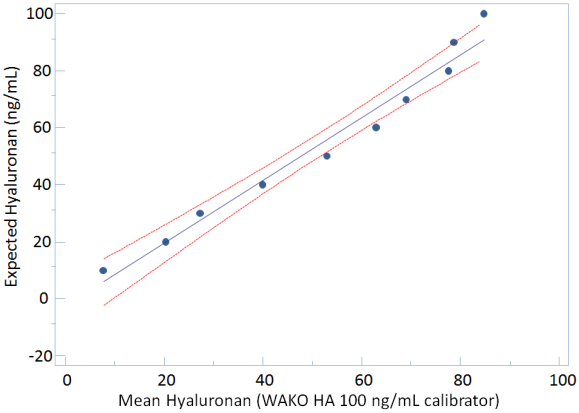


Repeated measurement (20x) of WAKO HA zero calibrator and serum sample pool (HA=16 ng/mL; 1:2 dilution (8 ng/mL); 1:4 dilution (4 ng/mL)); LOD=Limit of Detection.

Low end performance (CLSI EP17-A)

Serial dilution of recombinant HA concentrations provided linear response within a dilution range from 9:10 to 1:10 (1000 ng/mL, P <0.0001 and 100 ng/mL, P<0.0001). The recovery of HA was 94%-114% (1000 ng/mL) and 92%-130% (100 ng/mL), respectively.





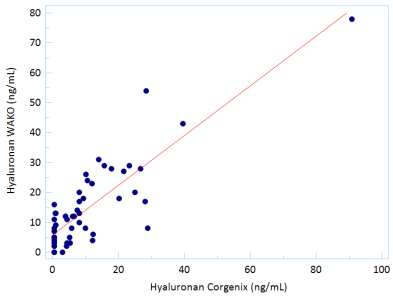
Repeated dilution measurement (2x) of WAKO HA 1000 ng/mL and 100 ng/mL; red lines 95%-confidence interval.

Linearity (CLSI EP06-A)

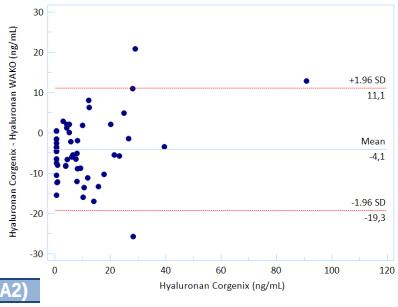
To run the intra-assay precision (20 replicates/day), 2 recombinant HA concentrations (100 and 400 ng/mL) and 1 serum pool (170 ng/mL) were prepared. The intra-assay precision study resulted in total coefficients of variation (CV) of 5.6%-6.1% for recombinant HA concentrations and 7.9% for serum pool. Inter-assay-precision yielded CVs of 2.2% and 3.9% (recombinant HA concentrations 100 and 400 ng/mL, respectively; 1 determination/day for 20 days).

Imprecision(CLSI EP05-A2)

Comparison of HA concentrations in healthy blood donors measured with the automated latex agglutination assay and the reference HEPASCORE HA-ELISA showed significant correlation (r=0.8525; P<0.0001).



Pearson correlation	Hyaluronan WAKO vs. Corgenix
Sample size	57
Correlation coefficient r	0.8525
Significance level	P<0.0001
95% Confidence interval for r	0.7610 – 0.9108



Bland & Altman plot: No systematic bias

Method comparison (CLSI EP09-A2)

Conclusion

Our data indicate that automated HA-latex agglutination assay presents excellent analytical performance and satisfactory correlation compared to reference method. It is easy to perform, accurate and enables the implementation of an automated HEPASCORE as routinely available non-invasive index of liver fibrosis.