

Hyaluronidase Treatment of Synovial Fluid in Biochemical Assays Using Roche Cobas Platforms

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Abstract

Synovial fluid (SF) is a difficult biological matrix to analyze because of its viscosity nature. This can lead to poor repeatability of tests and misinterpretation of results. Our study assessed the impact of SF treatment by hyaluronidase (HAse) to improve biochemical assays on Roche Cobas platforms included C-reactive protein (CRP), glucose (GLU), lactate dehydrogenase (LDH), lipase (LIP), rheumatoid factor (RF), total protein (TP), and uric acid (URIC). A string test was used to evaluate the level of viscosity. Normal SF will form a string of approximately 5 cm long before breaking. For the samples treated with HAse, the string test showed a total disappearance of viscosity. No significant interference was observed with the parameters investigated. The average percent recovery was within predefined acceptable limits (less than $\pm 10\%$ from the calculated ideal recovery). The intra-assay precisions determined in a single run ($n=10$), that is, 1.7% (CRP), 0.6% (GLU), 0.4% (LDH), 1.1% (LIP), 4.0% (RF), 1.2% (TP), and 0.8% (URIC) were close to the data established using Roche for serum and quality control matrix. Hyaluronidase treatment could be recommended for SF biochemical analysis to enhance the quality results.

Keywords: Biochemical assay; Hyaluronidase treatment; Matrix effect; Method validation; Synovial fluid

Synovial fluid (SF) is an ultrafiltrate or dialysate of plasma that contains levels of glucose and uric acid that are equivalent to plasma. SF, however, is at a lower level (approximately one-third) than that of plasma. This ultrafiltrate is combined with a mucopolysaccharide (hyaluronate) synthesized by the synovium.^[1] In some clinical contexts, biochemical analysis of SF samples can be performed by clinical laboratories. Several tests used in laboratory are standard processes designed for use with serum or plasma samples or urines samples. SF samples may resemble plasma in terms of protein and glucose concentrations, and may, at least in principle, be subject to interference because of this matrix difference. Hence, proper validation and con-

trol of methods used for body fluid samples have been made a condition of accreditation criteria of ISO 15189.^[2]

SF is very viscous because of its high concentration of polymerized hyaluronate (composed of alternating residues of β -D-(1-3) glucuronic acid and β -D-(1-4)-N-acetylglucosamine), especially if derived from healthy joints.^[3] This viscosity can cause problems when performing the test, especially when the sample is pipetted through the instrument or during the biochemical reaction. Some previous studies included hyaluronidase (HAse) treatment to liquefy the matrix SF.^[4] The HAse catalyzes the random hydrolysis of the 1-4 bond between N-acetyl-D-glucosamine and D-glucuronic acid in hyaluronic acid.

Cite this article as: Ilardo C, Herrero L, Barthes J. Hyaluronidase Treatment of Synovial Fluid in Biochemical Assays Using Roche Cobas Platforms. *Lokman Hekim Health Sci* 2021;1(3):114–118.

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E-mail: calogero.ilardo@labosud.fr **Submitted:** 24.08.2021 **Accepted:** 01.11.2021

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This study aimed to determine whether SF treatment with HAsE could improve the performances of analyses using Roche Cobas platforms.

Materials and Methods

The residual waste samples of SF used for validation of methods were collected in plain polypropylene or nonadhesive, non-gel blood collection tubes and stored at -20°C until analysis. The SF was centrifuged for 10 min at 2000 g, and the supernatants were pooled for testing experiments. The evaluation of freezing on SF matrix integrity was not conducted, but SF is an ultrafiltrate of plasma that keeps very well at this temperature.

SV Treatment

Before treatment, a string test was utilized to evaluate the level of viscosity. Normal SF will form a string approximately 5 cm long before breaking.^[5] The samples stored at -20°C did not interfere with the viscosity of SF.

HAsE reference H3506-1G was purchased from Sigma-Aldrich (St. Louis, MO, USA). HAsE was reconstituted from phosphate-buffered saline pH 7.2 (reference 444037E, VWR Chemicals, Radnor, PA, USA) to obtain an enzymatic solution at 67 mg/mL (approximately 46600 U/mL). The SF pooled was then treated by the following method: 495 μL of thawed SF and 5 μL of HAsE. The sample preparation was mixed and incubated for 15 min at 37°C . The mixture was centrifuged for 10 min at 2000 g for the removal of cell debris and protein aggregate.

Analytes Tested

Analytes tested included C-reactive protein (CRP), glucose (GLU), lactate dehydrogenase (LDH), lipase (LIP), rheumatoid factor (RF), total protein (TP), and uric acid (URIC). These studies were conducted on Roche Cobas 8000 chemistry modules (c502 and c702) using different technology: immunoturbidimetric assay for CRP and RF, UV method for GLU and LDH, and the colorimetric method for LIP, TP, and URIC. The involvement of these measurements in the diagnosis of joint diseases was described in Graff's Textbook of Routine Urinalysis and Body Fluids^[1] and in the literature review.^[6] The investigation of possible interferences between the assay methods and HAsE treatment was performed from 18 excess serum samples according to the French "Public Health Code."

Matrix and Assay Method Compatibility

To evaluate a possible interference of the SF matrix, five SF specimen was spiked with increasing concentrations of se-

rum specimen in the ratio of 75% SF + 25% spiking solution (by volume). $[X]_{\text{SF}}$ was the baseline analyte concentration in SF and $[X]_{\text{spike}}$ was the baseline analyte concentration in spiking material.

The "expected final concentration" was calculated as follows:

$$\text{Expected final concentration} = ([X]_{\text{SF}} \times 0.75) + ([X]_{\text{spike}} \times 0.25).$$

Each spiked SF sample was then tested in triplicate and an average final concentration $[X]_{\text{spiked average}}$ was determined. The "percent recovery" was calculated as follows:

$$\text{Percent recovery} = \left(\frac{[X]_{\text{spiked average}}}{\text{Expected final concentration}} \right) \times 100.$$

Within-run Accuracy

The within-run accuracy performance of SF treated was determined in a single run of 10 samples. The guideline on bioanalytical method validation^[7] specifications for within-run accuracy was used for comparison and CVs of <15% were considered acceptable.

Results

After HAsE treatment, the visual string test showed a total disappearance of viscosity (Fig. 1). The investigation of interferences due to pretreatment with HAsE showed no significant differences in concentration between samples when neat or HAsE treated (Table 1). The mean % difference was calculated as $[(\text{Neat} - \text{Hase})/\text{Neat}] \times 100$. For all interferences investigated, differences of less than $\pm 10\%$ were considered acceptable (Fig. 2). All analytes tested met threshold criteria for acceptable average percent recovery (less than $\pm 10\%$ from expected 100% recovery) (Table 1). Figure 3 shows the individual specimen percent recovery results for all analytes tested. An overall consistent recovery near the 100% goal is observed across analytes except for RF where the accuracy on one of the samples at 45UI/mL is <90% of the target but close to acceptable limits. Table 2 shows the intra-assay of each assay in an SF fluid matrix when compared with the stated performance of Roche QC material and serum on the Cobas. The SF intra-assay precisions determined in a single run ($n=10$), respectively 1.7% (CRP), 0.6% (GLU), 0.4% (LDH), 1.1% (LIP), 4.0% (RF), 1.2% (TP), and 0.8% (URIC) were close to the data established using Roche for serum and quality control matrix and remained below the requirements of the European Medicines Agency guideline on bioanalytical method validation (within-run CV value of <15%).^[7]

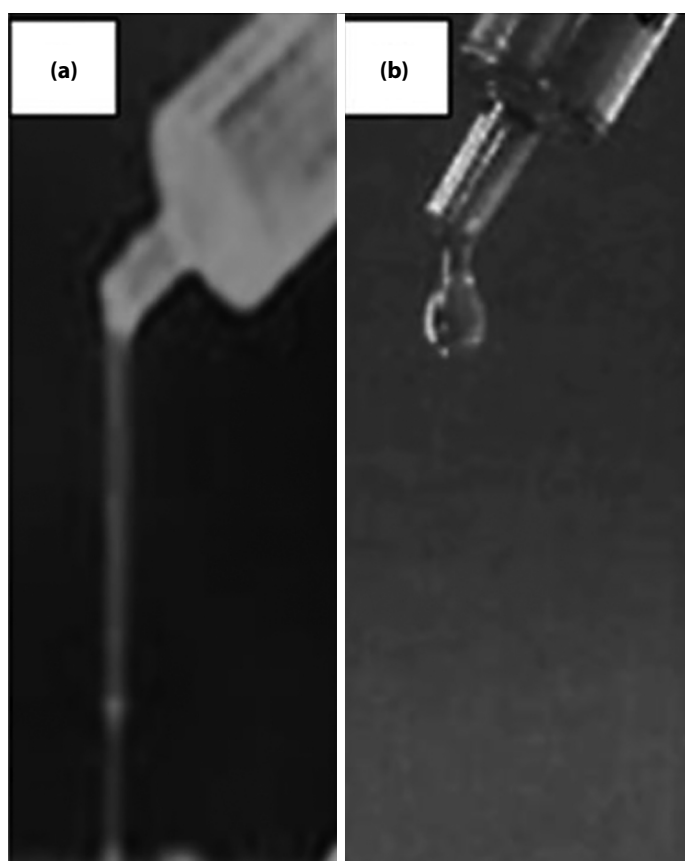


Figure 1. Physical examination of synovial fluid appearance before and after treatment by hyaluronidase (HAse). Legend: A=before treatment and B=after treatment.

Discussion

In the presence of viscous SF and absence of HAse treatment, the analysis of biochemical parameters is very often complicated or even impossible at the risk of clogging the sampling system of the instrument. The interaction between HAse and the analysis method is possible; therefore,

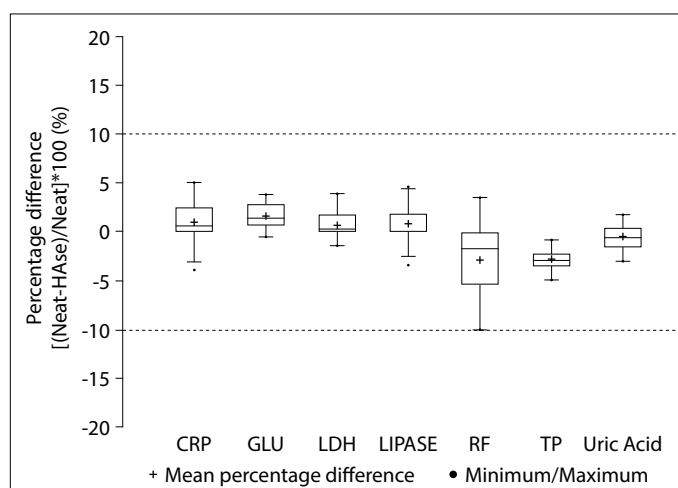


Figure 2. Boxplots of percentage difference between neat samples and samples treated by hyaluronidase (HAse).

laboratories should exercise caution and evaluate the impact of all pretreatment steps. The addition of HAse to SF samples causes a false increase in TP. Since HAse is an enzyme that is quantified by the TP assay, the results may be over-interpreted. However, given the low enzymatic mass provided during pretreatment (3.35 mg for 50 μ L of HAse), this overestimation remains limited and supportable (approximately 3%).

The use of a method for an application not validated by the manufacturer can be a real difficulty. Consequently, laboratories are left on their own to perform their own validation and derive their own specifications for acceptable criteria. According to the College of American Pathologists, laboratories may adopt method performance specifications that were originally derived from blood specimens if the laboratory can reasonably exclude the existence of matrix interferences.^[8]

Table 1. Hyaluronidase treatment interferences study and percent recovery of all spiked synovial fluid

Analyte	Samples Neat versus HAse treated						Average and SD % recovery (spiking) (n=5)
	Patient median (n=18)	Patient range	Slope	Intercept	Spearman correlation	Mann-Whitney test p value	
CRP (mg/L)	29.5	0.1–91	0.9667	0.3346	0.998	0.937	100.6 \pm 2.2
Glucose (mmol/L)	5.79	4.44–9.73	1.0023	0.1067	0.998	0.692	104.6 \pm 0.7
LDH (UI/L)	180	157–333	1.007	2.4512	0.982	0.772	104.1 \pm 0.6
Lipase (UI/L)	29.5	16–184	0.9876	0.1252	0.998	0.932	106.7 \pm 1.9
RF (UI/mL)	12.6	9.2–46.4	0.9857	0.5774	0.991	0.630	93.0 \pm 4.4
TP (g/L)	36.6	30.5–44.2	1.0149	0.5198	0.950	0.194	100.7 \pm 0.7
Uric acid (μ mol/L)	289	183–755	1.0021	0.586	1.000	0.950	103.6 \pm 0.4

SD: Standard deviation; CRP: C-reactive protein; LDH: Lactate dehydrogenase; RF: Rheumatoid factor; TP: Total protein.

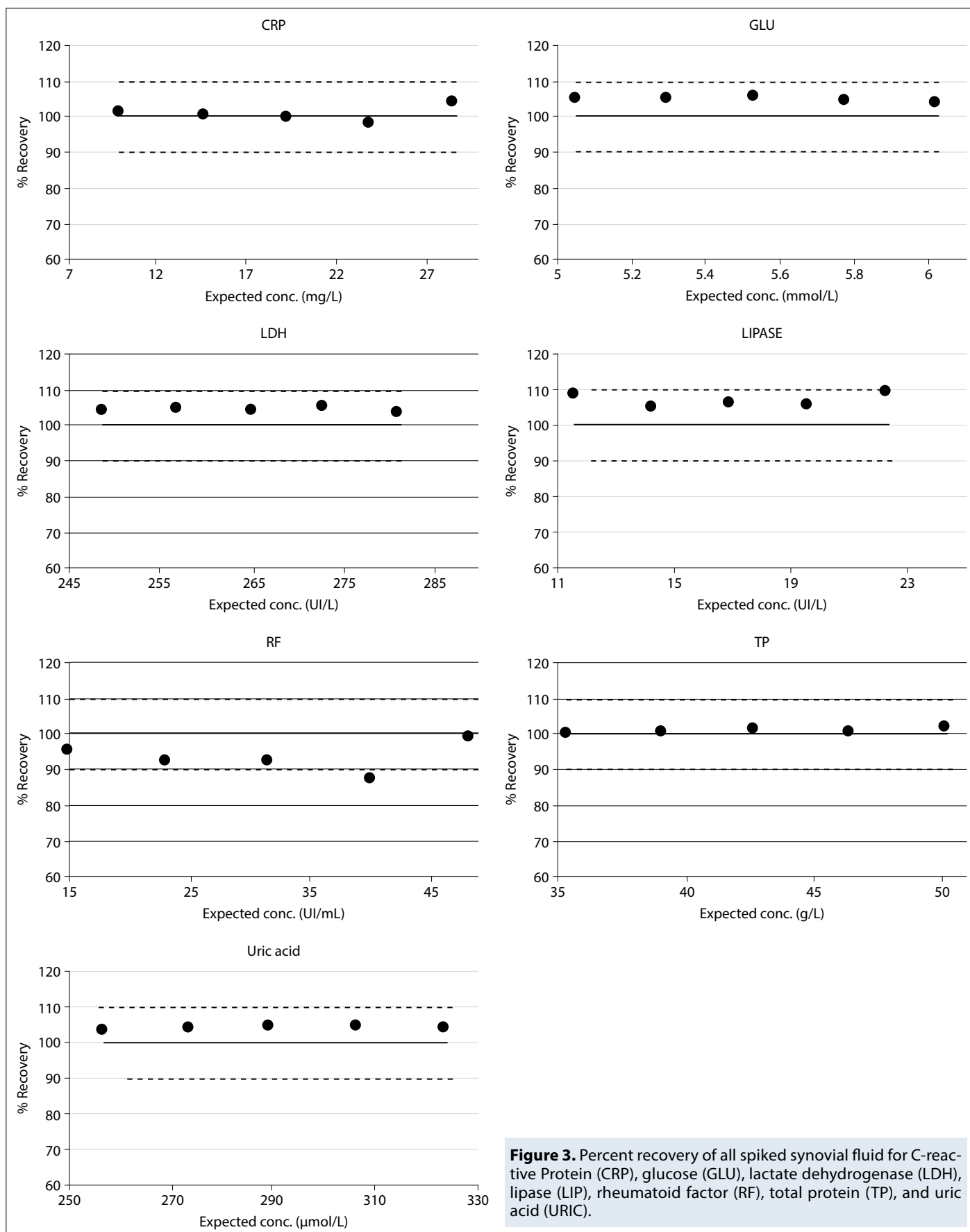


Figure 3. Percent recovery of all spiked synovial fluid for C-reactive Protein (CRP), glucose (GLU), lactate dehydrogenase (LDH), lipase (LIP), rheumatoid factor (RF), total protein (TP), and uric acid (URIC).

Table 2. Intra-assay precision for synovial fluid treated compared to manufacturer specifications for QC and serum precision

Analyte	Fluid type	N	Mean	SD	% CV
CRP (mg/L)	Synovial fluid	10	7.05	0.12	1.7
	QC control	21	9.6	0.06	0.6
	Serum	21	2.63	0.04	1.3
Glucose (mmol/L)	Synovial fluid	10	6.53	0.04	0.6
	QC control	21	5.17	0.04	0.8
	Serum	21	3.27	0.02	0.5
LDH (UI/L)	Synovial fluid	10	319.6	8.51	2.7
	QC control	21	274	1	0.4
	Serum	21	317	2	0.7
Lipase (UI/L)	Synovial fluid	10	12.3	0.48	3.9
	QC control	21	45.4	0.49	1.1
	Serum	21	12	0.28	2.3
RF (UI/mL)	Synovial fluid	10	8.7	0.35	4
	QC control	21	21.7	0.4	2.1
	Serum	21	12.6	0.4	3.3
TP (g/L)	Synovial fluid	10	40.3	0.48	1.2
	QC control	21	50	0.5	1
	Serum	21	52.9	0.5	0.9
Uric acid ($\mu\text{mol/L}$)	Synovial fluid	10	323	2.5	0.8
	QC control	21	255	2	0.6
	Serum	21	299	2	0.6

SD: Standard deviation; CRP: C-reactive protein; LDH: Lactate dehydrogenase; RF: Rheumatoid factor; TP: Total protein.

Conclusion

Our study showed that the enzymatic digestion of the large hyaluronic acid chains with HAse was useful for reducing viscosity to help improve the performance of the analysis of biochemical parameters using Roche Cobas. Other biological samples are rich in hyaluronic acid like amniotic fluid,^[9] and HAse could be tested for biochemical analysis.

Peer-review: Externally peer-reviewed.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Authorship Contributions: Concept: CI, LH, JB; Design: CI, LH; Supervision: JB; Fundings: CI; Materials: CI; Data Collection or Processing: CI; Analysis or Interpretation: CI, LH; Literature Search: CI; Writing: CI; Critical Review: CI, JB.

Conflict of Interest: None declared.

Financial Disclosure: The authors declared that this study received no financial support.

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