The Preservation Method and Timing on Accuracy of Manual Leukocytes Counts in Synovial Fluid

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Objectives: *To compare the efficacy of heparin and EDTA and determine the impact of time delays in stabilizing leukocyte counts in synovial fluid.*
Material and Method: *33 specimens were collected in heparin-preserved and EDTA-preserved containers. Total cell count was performed manually at 1 hour and 24 hours. Correlation between cell counts from both preservatives and the leukocyte number at 1 hour and 24 hours were analyzed by means of agreement measurement.*
Results: *There were good correlations between the leukocyte numbers from the specimens preserved by heparin and EDTA (ICC = 0.889, r = 0.879, P < 0.0001 at 1 hour and ICC = 0.822, r = 0.693, p < 0.0001 at 24 hour). At 24 hours, total cell counts from EDTA-preserved samples were comparable to those obtained at 1 hour (ICC = 0.985, r = 0.986, p < 0.0001) and were not different from those of the heparinized samples (ICC = 0.833, r = 0.751, p < 0.0001) but the ICC value was higher.*
Conclusion: *EDTA was as effective as heparin for preservation of synovial fluid. Therefore, it can be used routinely as a preservative of synovial fluid.*

Keywords: *Synovial fluid analysis, Leukocyte count, Heparin, EDTA, Preservation*

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Synovial fluid analysis is an essential laboratory investigation for the diagnosis of arthritis[1-5]. Total leukocyte counts of 200 to 2,000 cells/mm^3 are considered as the non-inflammatory fluid, typically found in osteoarthritis and traumatic arthritis. Cell counts between 2,000 and 50,000 cells/mm^3 are suggestive of inflammatory arthropathies such as infectious arthritis, crystal-induced arthritis and reactive arthritis[1]. Manual leukocyte count is traditionally recommended by using saline as a diluent to avoid protein precipitation[6].The synovial fluid samples should be examined immediately[1-2] or within a few hours after arthrocentesis[7]. If a delay cannot be avoided, keeping the joint fluid samples in heparinized containers at 4∞C is widely recommended, as a method to conserve the number of cell counts and crystal survival[7-10]. Salinas et al. demonstrated the advantage of EDTA as an anticoagulant to stabilize the leukocyte number in synovial fluid samples at 24 and 48 hours[11].

The objectives of our study were to compare the efficacy of two anticoagulants, heparin, and ethylene diamine tetra-acetic acid (EDTA), in this preservation of leukocyte count and to determine the impact of time delays on the accuracy of cell count in synovial fluid.

Material and Method

This study utilized a cross sectional study design. Thirty-three synovial fluid samples were obtained from patients with different causes of arthritis from patients attending the rheumatology unit at Siriraj Hospital, Bangkok, Thailand, between July 2005 and December 2005. A quantity of 4 ml for each samples were divided and placed in two tubes containing sodium heparin and EDTA. These samples were immediately delivered to the laboratory office in the Depart-

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ment of Clinical Pathology after arthrocentesis. Manual leukocyte counts were performed by a well-trained technician. Synovial fluid with high leukocyte counts were diluted with saline and were analyzed using a haemacytometric chamber while those specimens with low cell counts were examined undiluted using the standard method similar to the cerebrospinal fluid cell counts. The remaining fluid samples were stored in a refrigerator at 4°C for 24 hours and were re-examined. The leukocyte numbers from heparinized and EDTA-preserved specimens were compared. To determine the impact of time elapse on the accuracy and stability of leukocyte counts, the leukocyte number examined at 1 hour and 24 hour were evaluated.

Synovial fluid samples of less than 4 ml and those that could not be transferred to the laboratory office within 1 hour after extraction were excluded from the study.

Statistics

Imprecision studies

A well-trained technician performed and repeated the process of leukocyte count 20 times on the same day using two synovial fluid samples with high and low leukocyte counts to determine the intra-observer variation. The coefficient of variation (CV) was calculated.

Agreement measurement

To eliminate the inter-observer variation, each of the specimens transferred with- and without delay was examined by the same technician.

Pearson's correlation coefficient was applied to determine the influence of the preservation method with heparin and EDTA and the time-dependent effect on the total leukocyte count. An Intraclass Correlation Coefficient (ICC) of more than 0.8 indicated the agreement of parameters.

The SPSS for windows, version 11.5, was utilized for the statistical analysis and data management system.

Results

Thirty-three synovial fluid samples from thirty-three patients were included in this study. The baseline characteristics are described in Table 1. The presumptive diagnosis is shown in Fig. 1. Most patients had preexisting medical conditions such as diabetes, hypertension and chronic kidney disease. The mean time elapsed prior to arthrocentesis was 12.39 days. Monoarthritis and oligoarthritis were the most common presenting symptoms. The synovial fluid specimens were in the inflammatory range in 72.7% of cases.

Table 1. Baseline characteristics of patients and synovial fluid samples

Variable	Number (%)
Patients	
Sex - Male : Female	16:17 (48.5:51.5)
Age : yr.	53.9 ± 16
Underlying disease	
Diabetes mellitus	14 (42.4)
Hypertension	3 (9.1)
Chronic kidney disease	3 (9.1)
Dyslipidemia	1 (3)
Others	12 (36.4)
Indication for arthrocentesis	
Diagnosis	32 (97)
Treatment	1 (3)
Number of joint involvement	
Monoarthritis	19 (57.4)
Oligoarthritis	13 (39.3)
Polyarthritis	1 (3)
Synovial fluid	
Gross appearance	
- Clear	16 (48.5)
- Turbid	16 (48.5)
- Pus	1 (3)
Color	
- Straw	30 (90.9)
- Bloody	1 (3)
- Others	2 (6.1)
Leukocyte count	
- inflammatory (2,180-124,000 cell/mm^3)	24 (72.7)
- non-inflammatory (70-1,280 cell/mm^3)	9 (27.3)
Presence of crystal	
- Present	21 (63.6)
- Absent	12 (36.4)
Presence of organism	
Method : Gram staining	
- Positive for organism	2 (6.1)
- Negative for organism	31 (93.9)
Method : Culture	
- Growth	3 (9.1)
- No growth	30 (90.9)

Imprecision study

The intra-observer variation of the leukocyte count is presented in Table 2. The within-day variation for total cell count from the specimen with low-level leukocyte numbers was less than that of the high-level

Table 2. The intra-observer imprecision study

Synovial fluid : leukocyte count level	Mean ± SD	Coefficient of variation (CV) %
Low level	1,564 ± 173.34	11.08
High level	156,050 ± 62,753.85	40.21

Table 3. Leukocyte counts obtained from heparin- and EDTA-preserved synovial fluid at 1 hour and 24 hours

Synovial fluid	Mean (cell/mm^3)	Median (cell/mm^3)	Standard deviation	Range (cell/mm^3)
Heparin-preserved specimen at 1 hour	30,570.82	21,000	34,096.22	70-124,000
Heparin-preserved specimen at 24 hours	39,520.17	26,500	47,739.86	75-178,000
EDTA-preserved specimen at 1 hour	37,330.00	22,750	52,303.11	70-265,600
EDTA-preserved specimen at 24 hours	36,237.40	25,300	45,982.75	100-214,400

one, indicating better accuracy when this traditional method was performed in the non-inflammatory type of synovial fluid.

Leukocyte count

1. Comparison of leukocyte count numbers between heparinized sample and EDTA-preserved samples

Good correlation between the leukocyte numbers of both heparin and EDTA preservation at 1 and 24 hours are demonstrated in Figures 2 and 3 (ICC = 0.889, r = 0.879, P < 0.0001 at 1 hour and ICC = 0.822, r = 0.693, p < 0.0001 at 24 hours).

2. The effect of time delay on the number of leukocyte count numbers in synovial fluid

At 24 hours, the total leukocyte count numbers achieved from heparinized synovial fluid samples was not different from those at 1 hour (ICC = 0.833, r = 0.751, p < 0.0001), as shown in Figure 4. This finding was similar to the leukocyte numbers obtained from

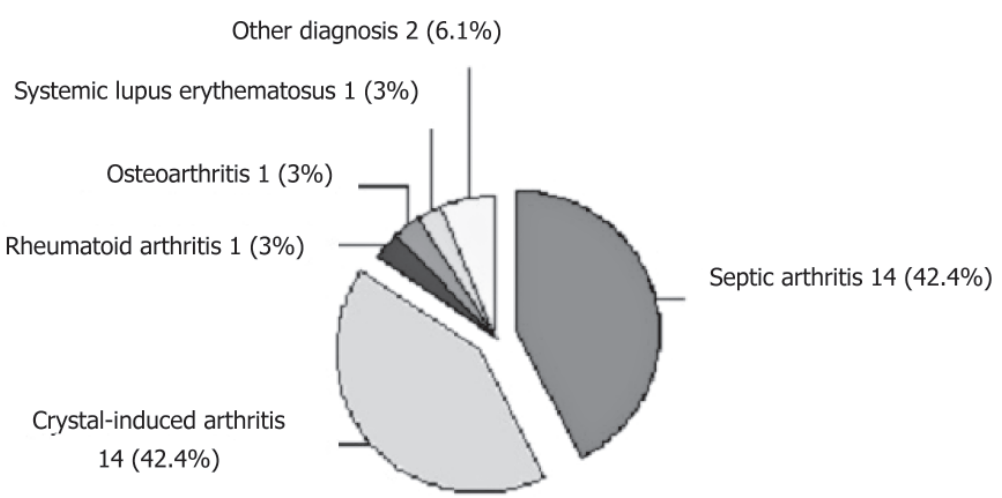

Fig. 1 Presumptive diagnosis of the causes of arthritis

EDTA preserved sample (ICC = 0.985, r = 0.986, p < 0.0001), as shown in Fig. 5.

A summary of leukocyte numbers counted from heparin- and EDTA-preserved synovial fluid at 1 hour and 24 hours is shown in Table 3.

Discussion

Synovial fluid analysis has long been recognized as one of the most useful tests for the diagnosis and assessment of arthritis[1-5]. The leukocyte number in the synovial fluid provides an important clue to differentiate the inflammatory joint disease from the non-inflammatory one[1-3]. The total nucleated cell count is measured using a hematological counting chamber in a standard way in the heparin-preserved specimens[1-3]. In the study conducted by Schumacher, a decrease in leukocyte count was observed as early as one hour after joint fluid aspiration[12]. For that reason, synovial fluid analysis should be considered as an emergency[12]. However, there have been a number of later studies demonstrating that interpretable results can still be attained after 48-72 hours[11]. In a small pilot study by Salinas et al, the leukocyte count performed after keeping the EDTA preserved synovial fluid for 48 hours was more stabilized than that of a heparin preserved specimen[11].

Although manual cell count in synovial fluid is the procedure that is generally recommended[9], attempts have been made to automate cell counting. In the previous report by Vincent et al., the use of automated cell counters was discouraged because of the possible error caused by fat droplets and possible cell damage by the machine. Sugiuchi et al. recently developed a new method of pretreatment with hyaluronidase, then counted the total cell number by means of an automated hematology analyzer. The result showed good correlation with that obtained by manual counting[13]. Automated cell count may offer advantages of higher precision, accuracy and time saving [11]. In our study, we utilized the traditional manual leukocyte count by a well-trained technician. To determine the precision of manual technique, the Coefficient of Variation (CV) was calculated. The intra-observer variation in the synovial fluid with low cellularity was less (CV = 11.08%) than that of the higher cell count number (CV = 40.21%). It indicates better accuracy when this reference method is performed with the non-inflammatory synovial fluid. Our results are

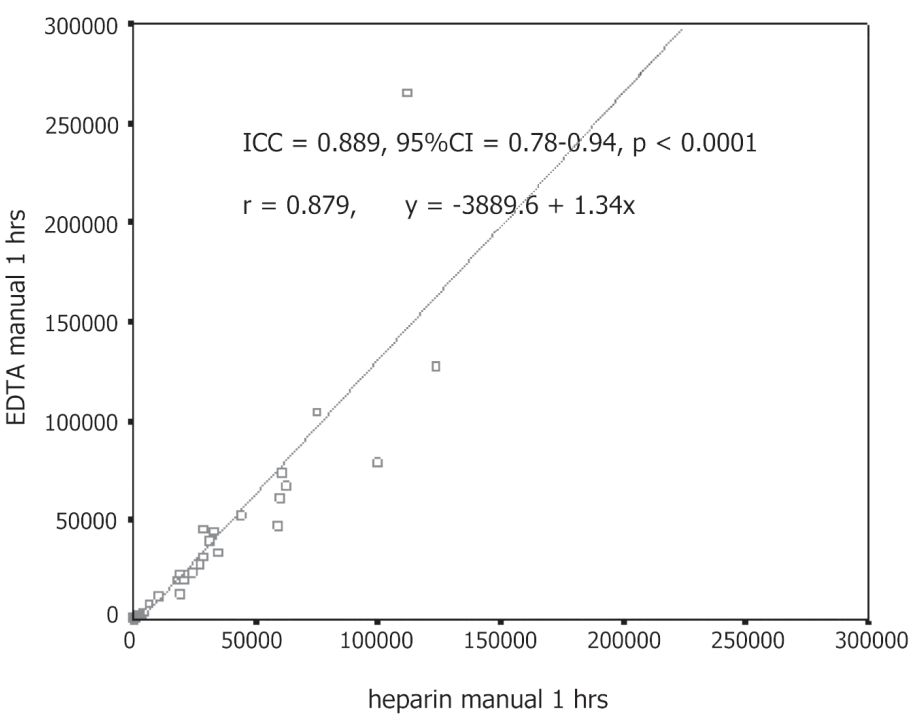

Fig. 2 Correlation between leukocyte counts obtained from heparin and EDTA preserved synovial fluid at 1 hour

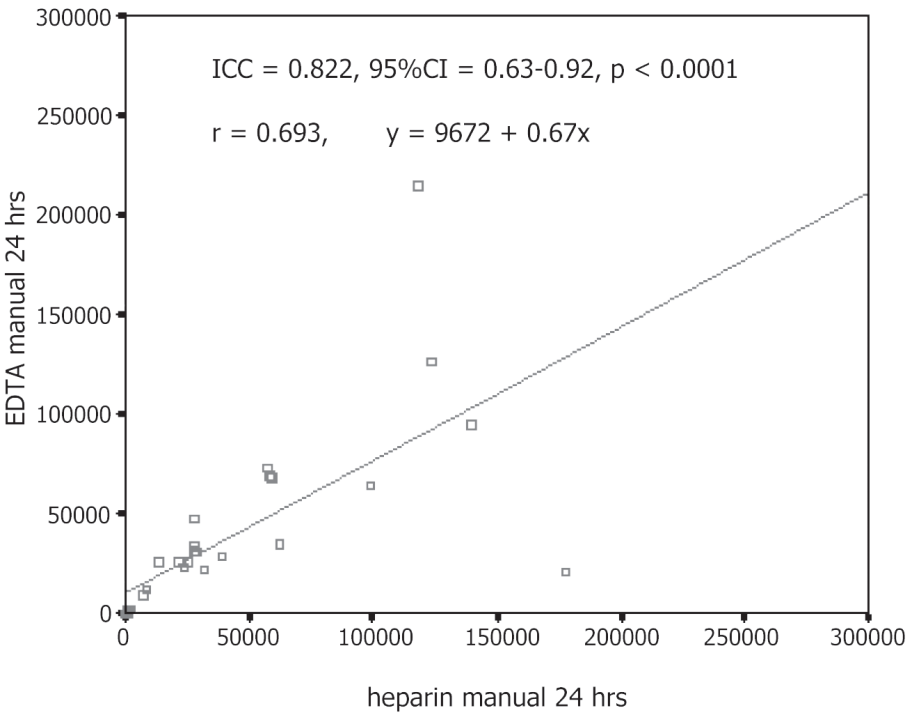

Fig. 3 Correlation between leukocyte counts obtained from the heparin and EDTA preserved synovial fluid at 24 hours

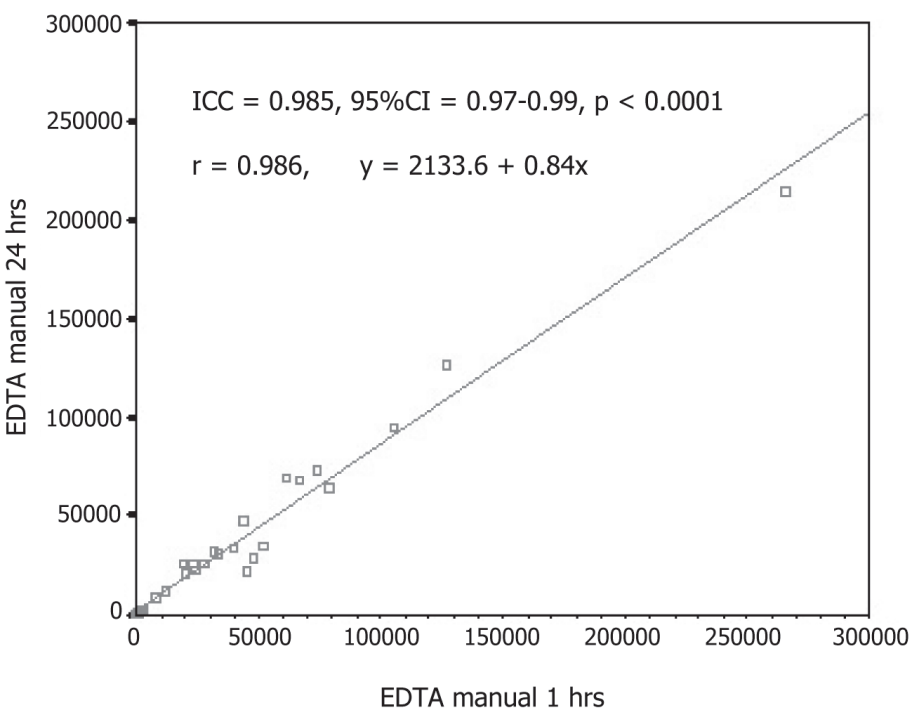

Fig. 4 Correlation between leukocyte counts obtained from heparin preserved synovial fluid specimens at 1 and 24 hours

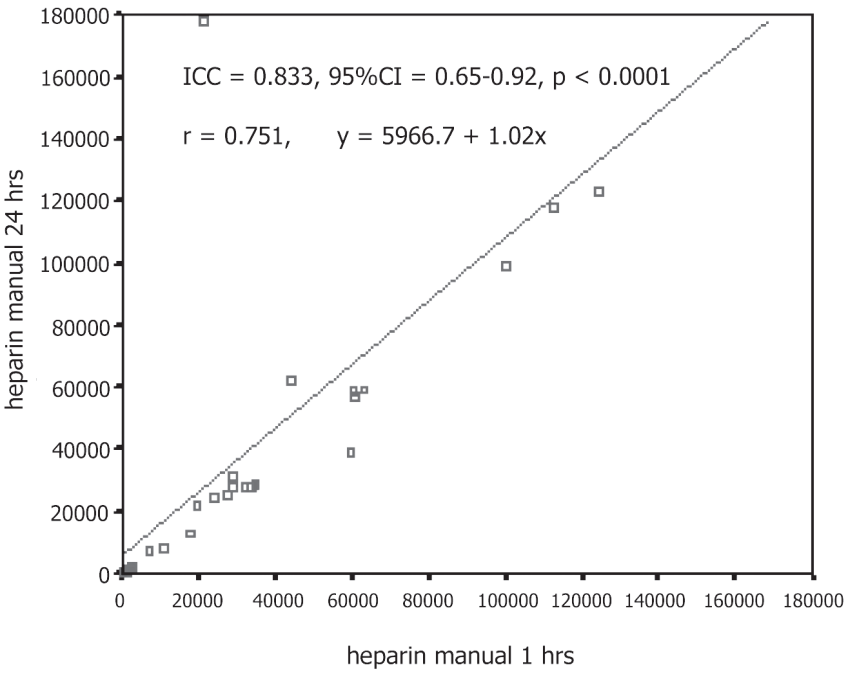

Fig. 5 Correlation between leukocyte counts obtained from the EDTA preserved synovial fluid specimens at 1 and 24 hour

different from those obtained by Schumacher et al. that found a higher CV value of 20-62% in the fluid specimens with low cell count (< 300 cells/mm^3) and a lower value of 1-18% in the samples of more than 1,500 cell/mm^3 [12]. This discrepancy may be explained by the difference in the number of synovial fluid specimens used for this purpose. Only two specimens with low and high cellularity (1,564 and 156,050 cell/mm^3) were analyzed in our study while four separate samples with a lower range of cell numbers (200-40,000 cell/mm^3) were analyzed in Schumacher's study. The diluted method with saline normally used in synovial fluid with high cell counts might interfere with the result of the leukocyte number. The variation among the cell counters was also reported earlier in other studies[11,12,14]. It should be remembered that the imprecision evaluated in our study depended on the performance of only one well-trained technician. In daily practice, manual leukocyte count is generally accomplished by a team of trained medical technicians. Therefore, the variation is expected to be larger. Regular monitoring and quality control can improve the accuracy of the results.

Leukocyte counts at 1 hour and 24 hours from EDTA-preserved specimens were highly correlated with those obtained by heparin-preserved specimen (ICC = 0.889 and 0.822 respectively). The cell counts performed after keeping the EDTA-and heparin-preserved synovial fluid for 24 hours were very close to the values obtained immediately within 1 hour. A higher correlation was noted surprisingly in the synovial fluid cell counts using EDTA as an anticoagulant (ICC = 0.985) while it was 0.833 in the heparinized samples. However, a small decrease in the total leukocyte number occurred in the delayed examination without statistical significance. Our outcomes agree with those reported by Salinas et al.[11]. In two previous studies, a significant decrease in leukocyte counts were found in the specimens kept with heparin[12,15]. EDTA is the standard anticoagulant normally used in automated blood cell counting due to its ability to prevent clot formation. EDTA offers greater advantages than heparin because of its lower cost, easy reproducibility, and preparation. To differentiating the leukocytes with Wright staining technique, EDTA-preserved synovial fluid does not produce dark-blue discoloration on the background. Therefore, the ability to distinguish cell types is more comprehensible than in the heparin-preserved samples. Our data strongly suggests that EDTA is more suitable than heparin to be used as a routine preservative of the synovial fluid.

Conclusion

The present study showed that EDTA was as effective as heparin in stabilizing total leukocyte numbers at 1 hour and 24 hours, and provided results that are more accurate with the delayed specimens. Therefore, as an alternative, EDTA can be used to preserve synovial fluid.

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การศึกษาผลของวิธีการเก็บรักษาน้ำไขข้อและระยะเวลาที่มีต่อความถูกต้องแม่นยำของการตรวจนับเซลล์เม็ดเลือดขาวโดยบุคลากรทางห้องปฏิบัติการ

อัจฉรา กุลวิสุทธิ์, อำไพวรรณ รุ่งบรรณพันธุ์, วันรัชดา คัชมาตย์, วิมล ชินสว่างวัฒนกุล

วัตถุประสงค์: *เพื่อทราบผลของการใช้สารเฮปารินและอีดีทีเอในการรักษาปริมาณของเซลล์เม็ดเลือดขาวในน้ำไขข้อ และประเมินผลกระทบของระยะเวลาในการเก็บรักษาน้ำไขข้อ ที่มีต่อปริมาณของเม็ดเลือดขาว*

รูปแบบการวิจัย: *Cross-sectional study*

วัสดุและวิธีการ: *ศึกษาน้ำไขข้อจำนวน 33 ตัวอย่าง โดยนำมาเก็บในภาชนะที่ใส่สารเฮปารินซึ่งเป็นสารมาตรฐาน และสารเอทธีลีนไดอามีนเตตราอะซีติกแอซิด (อีดีทีเอ) ตรวจนับจำนวนเซลล์เม็ดเลือดขาวโดยบุคลากรผู้ชำนาญทางห้องปฏิบัติการ ณ เวลา 1 ชั่วโมง และ 24 ชั่วโมง เปรียบเทียบความแตกต่างของจำนวนเซลล์ในน้ำไขข้อที่เก็บรักษาโดยสารทั้ง 2 ชนิด และจำนวนเซลล์ใน 2 ช่วงเวลา*

ผลการศึกษา: *จำนวนเม็ดเลือดขาวที่นับได้จากน้ำไขข้อซึ่งเก็บรักษาด้วยสารอีดีทีเอมีความสัมพันธ์สอดคล้องที่ดีกับจำนวนที่ได้จากน้ำไขข้อซึ่งเก็บรักษาด้วยสารเฮปาริน ใน 2 ช่วงเวลา (ICC = 0.889, r = 0.879, p < 0.0001 ณ เวลา 1 ชั่วโมง และ ICC = 0.822, r = 0.693, p < 0.0001 ณ เวลา 24 ชั่วโมง) จำนวนเม็ดเลือดขาวที่นับได้จากน้ำไขข้อที่เก็บรักษาด้วยอีดีทีเอ ณ เวลา 1 ชั่วโมง และ 24 ชั่วโมงมีความสัมพันธ์สอดคล้องกันดี (ICC = 0.985, r = 0.986, p < 0.0001) เช่นเดียวกับน้ำไขข้อที่เก็บรักษาด้วยสารเฮปาริน (ICC = 0.833, r = 0.751, p < 0.0001)*

สรุป: *สารอีดีทีเอมีประสิทธิภาพในการรักษาจำนวนเม็ดเลือดขาวในน้ำไขข้อได้ดีเทียบเท่ากับสารเฮปาริน สามารถนำมาใช้ทดแทนสารเฮปารินในการเก็บรักษาน้ำไขข้อได้*