

A COMPARISON OF AUTOMATED COMPLETE BLOOD COUNT PARAMETERS AND HEMOLYSIS OCCURRENCE FROM VACUUM AND NON-VACUUM K₂EDTA TUBES

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Abstract

Background: The preanalytical factors, including tube type and storage duration, may influence CBC results and hemolysis. Nowadays, blood collection using K₂EDTA, both vacuum and non-vacuum tubes, is widely utilized in clinical laboratories for complete blood count (CBC) analysis. However, the impact of vacuum-induced negative pressure and the anticoagulant-to-blood ratio in under-filled tubes on sample integrity remains a concern.

Objective: This study aimed to compare the performance of K₂EDTA vacuum and non-vacuum blood collection tubes, including under-filled non-vacuum tubes, in automated CBC parameters and to assess hemolysis occurrence at different time points.

Methods: Blood samples from 30 healthy subjects were collected into three different K₂EDTA blood collection tubes: vacuum, non-vacuum, and non-vacuum tubes with under-filled volumes. CBC parameters, including RBC, Hb, Hct, MCV, MCH, MCHC, RDW, WBC, PLT, and MPV, were analyzed at 0 hours, 3 hours, and 24 hours post-collection. Hemolysis was assessed via spectrophotometry at 540 nm.

Results: No statistically significant differences were observed in CBC parameters among the three groups at 0 hours. However, significant differences occurred at 3 hours post-collection, including WBC and PLT in vacuum tubes, MCV in non-vacuum tubes, RDW in under-filled tubes, and MPV in all tube types. At 24 hours, all tube types showed significant changes in MCV, MCHC, RDW, and MPV, while RBC showed only changes in vacuum tubes. The highest hemolysis levels occurred in vacuum tubes at 0 hours, whereas under-filled tubes exhibited increased hemolysis at 3 and 24 hours.

Conclusion: The results indicated that all three tube types are suitable for hematological analysis if processed within 3 hours. However, the risk of hemolysis in vacuum tubes should be carefully considered, particularly in individuals with suspected hemolysis.

Keywords: vacuum K₂EDTA tubes, non-vacuum K₂EDTA tubes, under-filled collection tubes, CBC analysis, hemolysis level

J Southeast Asian Med Res 2025; 9: e235

<https://doi.org/10.55374/jseamed.v9.235>

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Received: 18 March 2025

Revised: 29 April 2025

Accepted: 2 May 2025

Introduction

The preanalytical phase of laboratory testing involves several critical steps: specimen collection, transportation, and preparation before analysis.⁽¹⁾ Among these, the specimen collection step is prone to errors. Incorrect procedures during this phase can result in low-quality and unsuitable samples.

Dipotassium ethylene diamine tetraacetic acid (K₂EDTA) and tripotassium ethylene diamine tetraacetic acid (K₃EDTA) are standard anticoagulants used in blood collection tubes for hematological analysis, including complete blood count (CBC), reticulocyte count, and hemoglobin type and quantity analysis. EDTA acts as an anticoagulant by binding to calcium, essential for coagulation reactions. It also effectively maintains blood cell condition, number, appearance, and size. Some studies have reported slight differences in hemoglobin levels between K₂EDTA and K₃EDTA tubes, possibly due to the solution form of K₃EDTA.^(2, 3) K₂EDTA is commonly used in the United States, while K₃EDTA is more popular in European countries and Japan. In Thailand, both are used, depending on hospital procurement policies.

In clinical laboratories, vacuum and non-vacuum blood collection tubes are routinely used, each with specific advantages and limitations. The vacuum system relies on the principle of the blood pressure entering the tube. The vacuum inside the tube helps draw blood when inserted into a vein, making the collection process more efficient. Non-vacuum systems, often using syringe transfer or aspiration, are more flexible in volume control.⁽⁴⁾ Each system may influence preanalytical variables such as clot formation, hemolysis, and anticoagulant-to-blood ratio, affecting test accuracy.

Vacuum blood collection tubes are typically used for larger blood volumes and tests requiring a specific volume. In contrast, non-vacuum tubes

are often used for smaller volumes and tests that do not require precise measurements. Using vacuum blood collection tubes is now internationally recognized, significantly enhancing the diagnostic process's accuracy, safety, convenience, and speed. Additionally, they are endorsed by at least two major standard-setting organizations: the Clinical and Laboratory Standards Institute (CLSI),⁽⁵⁾ and the International Organization for Standardization (ISO).⁽⁶⁾

The concept of vacuum blood collection tubes was initially introduced in 1945 by Joseph Kleiner, as reported by Weikart et al.⁽⁷⁾ However, various issues have been reported concerning the use of vacuum blood collection tubes, particularly regarding environmental factors that may affect them. These factors include the materials used in tube production,⁽⁷⁾ the amount of residual air in the tube, and the internal air pressure at manufacture.⁽⁸⁾ Higher internal pressure can lead to an under-filling of the vacuum tube. Such under-filled volumes in non-vacuum tubes may occur in pediatric patients or individuals with fragile veins, and precise blood collection is difficult. Under-filled tubes result in a disproportionate ratio of anticoagulant to blood, which may impact the hematological testing,^(9, 10) particular enzymes,^(11, 12) and molecular biology testing.⁽¹³⁾

The hemolyzed specimen is a preanalytical issue in clinical laboratories. *In vitro* hemolysis may occur during the sample collection step, leading to the rejection of the specimen or interfering with laboratory results.⁽¹⁴⁾ Previous studies have demonstrated that blood collection tubes and methods impact the probability of hemolysis. Vacuum tubes have been associated with higher hemolysis rates due to residual negative pressure, particularly in pediatric⁽⁸⁾ or catheter-based collections.^(15, 16) Non-vacuum tubes by syringe or aspiration techniques demonstrate lower hemolysis rates.^(17, 18) *In vitro* hemolysis

causes the release of intracellular components, dilution effects, and interference with analysis techniques, significantly affecting the interpretation of laboratory results.⁽¹⁹⁾

Choosing the appropriate blood collection equipment and devices ensures accurate and reliable analysis and interpretation. This accuracy directly impacts the diagnosis and treatment of patients. Therefore, this study aims to evaluate the performance of K₂EDTA vacuum and non-vacuum blood collection tubes, including under-filled non-vacuum tubes, in automated CBC parameters and to assess hemolysis occurrence at different time points.

Methods

Collection of Diagnostic Blood Specimens

This study was approved by the Institutional Review Board of the Royal Thai Army Medical Department (IRBRTA 1673/2566). A group of 30 healthy subjects, 20-80 years of age, was used for analysis. Subjects over this age range were excluded. Of the 9 mL of whole blood, 3 mL was dispensed into K₂EDTA vacuum tubes (group A), 3 mL and 2 mL were collected in K₂EDTA non-vacuum tubes (group B and group C, respectively), and 1 mL was stored in a no-additive tube as a blank for hemolysis testing. Group C was filled with 2 mL of blood into 3 mL non-vacuum tubes, lower than the recommended fill volume.

Complete Blood Count Analysis

Each group was analyzed at three time points at room temperature (RT): 0 (within 30 minutes), 3, and 24 hours post-collection. Complete blood count (CBC) analysis was performed using an automated 5-part differential analyzer, model LH 780 Hematology Analyzer (Beckman Coulter, Inc., California, USA). The CBC analysis included ten parameters: erythrocytes (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), leukocytes (WBC), platelets (Plt), and mean platelet volume (MPV).

Hemolysis testing

After CBC analysis at each time point, 300 microliters of blood were separated for hemoly-

sis testing. Plasma was isolated by centrifugation and stored at -20 °C before measuring the optical density (OD) at 540 nm using a visible spectrophotometer. Serum from the non-additive tube was used as a blank, and blood mixed with distilled water was used as the 100% hemolysis control.

Statistical Analysis

Statistical analyses were conducted using STATA/BE, version 18.0 (StataCorp LLC, College Station, TX, USA). The significance of the differences among the three groups of K₂EDTA tubes for diagnostic blood specimen collection was assessed using One-way ANOVA. Repeated measures ANOVA was performed to compare the differences within groups between baseline (0 hours) and 3 hours post-collection, as well as between baseline and 24 hours post-collection. The mean difference was calculated by subtracting the mean at 3 or 24 hours from the mean at 0 hours. A *p*-value of < 0.05 was considered statistically significant.

Results

The study evaluated differences in CBC parameters and hemolysis percentage across three types of K₂EDTA blood collection tubes: vacuum tubes (A), non-vacuum tubes (B), and non-vacuum tubes with underfilled volumes (C). This study included 30 healthy subjects, 12 males (40%) and 18 females (60%), with an average age of 34 ± 9 years.

The results of the CBC analysis in groups A, B, and C are presented in **Table 1**. No statistically significant differences were observed among the three groups at 0 hours. The values obtained at 0 hours were used as a baseline to assess the changes observed at 3 and 24 hours. However, significant changes were observed within each group at 3 hours post-collection, including WBC and platelet counts in group A (*p* = 0.009 and *p* = 0.048, respectively), MCV in group B (*p* = 0.026), and RDW in group C (*p* = 0.005).

At 24 hours post-collection, significant changes were observed in MCV, MCHC, RDW, and MPV within all three groups (**Figure 1**). At the same time, RBC count showed a statistically significant change only in group A (*p* = 0.013). Over time,

Table 1. The mean of CBC parameters was at 0 hours for the three groups of K₂EDTA tube (highlighted row), and the mean differences were between the CBC parameters of the three groups at different time points.

	A	p-value within group A	B	p-value within group B	C	p-value within group C	p-value (compare three groups)
RBC count x(10 ⁶ /uL)	4.77±0.7	4.77±0.68	4.76±0.68	0.995			
diffRBC (3 hr)	-0.02±0.08	0.207	0±0.06	0.953	-0.04±0.11	0.071	0.221
diffRBC (24 hr)	-0.03±0.05	0.013*	-0.01±0.07	0.382	-0.03±0.17	0.319	0.780
Hb (g/dL)	12.31±2.57	12.71±1.55	12.69±1.5	0.663			
diffHb (3 hr)	-0.44±2.26	0.291	0±0.16	1.000	-0.07±0.24	0.136	0.375
diffHb (24 hr)	-0.48±2.26	0.257	0.16±1.24	0.486	-0.07±0.29	0.217	0.253
Hct (%)	39.51±4.58	39.53±4.59	39.38±4.43	0.990			
diffHct (3 hr)	-0.25±0.71	0.291	-0.07±0.56	1.000	-10.72±56.55	0.136	0.356
diffHct (24 hr)	-1.35±0.71	0.257	-1.2±0.8	0.486	-1.41±1.56	0.217	0.738
MCV (fL)	83.58±8.99	83.63±8.92	82.68±10.18	0.907			
diffMCV (3 hr)	-0.13±0.39	0.076	-0.17±0.39	0.026*	-0.98±4.94	0.286	0.434
diffMCV (24 hr)	-2.45±0.9	<0.001*	-2.3±1.17	<0.001*	-3.20±5.1	0.002*	0.348
MCH (pg)	26±6.02	26.93±3.38	26.98±3.43	0.631			
diffMCH (3 hr)	-0.87±5.22	0.371	0.01±0.24	0.880	0.08±0.31	0.191	0.408
diffMCH (24 hr)	-0.97±5.21	0.315	0.44±2.65	0.367	0.06±0.56	0.540	0.251
MCHC (g/dL)	32.14±0.97	32.13±0.82	32.21±0.94	0.936			
diffMCHC (3 hr)	0.1±0.62	0.397	0.06±0.33	0.307	0.11±0.37	0.117	0.921
diffMCHC (24 hr)	0.85±0.57	<0.001*	1.39±2.96	0.015*	0.91±0.78	<0.001*	0.438
RDW (%)	14.58±1.31	14.65±1.37	14.5±1.3	0.908			
diffRDW (3 hr)	-0.08±0.52	0.390	-0.05±0.29	0.356	-0.12±0.22	0.005*	0.743
diffRDW (24 hr)	-1.51±0.6	<0.001*	-1.54±0.44	<0.001*	-1.52±0.44	<0.001*	0.977
WBC count x (10 ⁶ /uL)	7.38±2.06	7.49±2.24	7.39±2.17	0.978			

Table 1. The mean of CBC parameters was at 0 hours for the three groups of K₂EDTA tube (highlighted row), and the mean differences were between the CBC parameters of the three groups at different time points. (Cont.)

	A	p-value within group A	B	p-value within group B	C	p-value within group C	p-value (compare three groups)
diffWBCcount (3 hr)	-0.24±0.46	0.009*	-0.03±0.34	0.633	-0.18±0.5	0.062	0.180
diffWBCcount (24 hr)	-0.1±0.38	0.173	-1.99±12	0.372	-0.11±0.43	0.183	0.481
Pltcount x (10 ⁹ /uL)	276.17±55.93		273.5±55.44		273.9±55.72		0.980
diffPltcount (3 hr)	4.9±12.98	0.048*	3.47±10.92	0.093	4.17±25.37	0.376	0.952
diffPltcount (24 hr)	6.37±17.95	0.062	4.83±23.78	0.275	7.00±21.04	0.079	0.920
MPV (fL)	8.47±1.26		8.44±1.3		8.46±1.29		0.996
diffMPV (3 hr)	-0.30±0.22	<0.001*	-0.36±0.31	<0.001*	-0.35±0.26	<0.001*	0.684
diffMPV (24 hr)	0.32±0.37	<0.001*	0.26±0.52	0.011*	0.37±0.47	<0.001*	0.615

A: vacuum K₂EDTA tubes, B: non-vacuum K₂EDTA tubes, C: non-vacuum K₂EDTA tubes with under-filled volumes

*p < 0.05 is significant

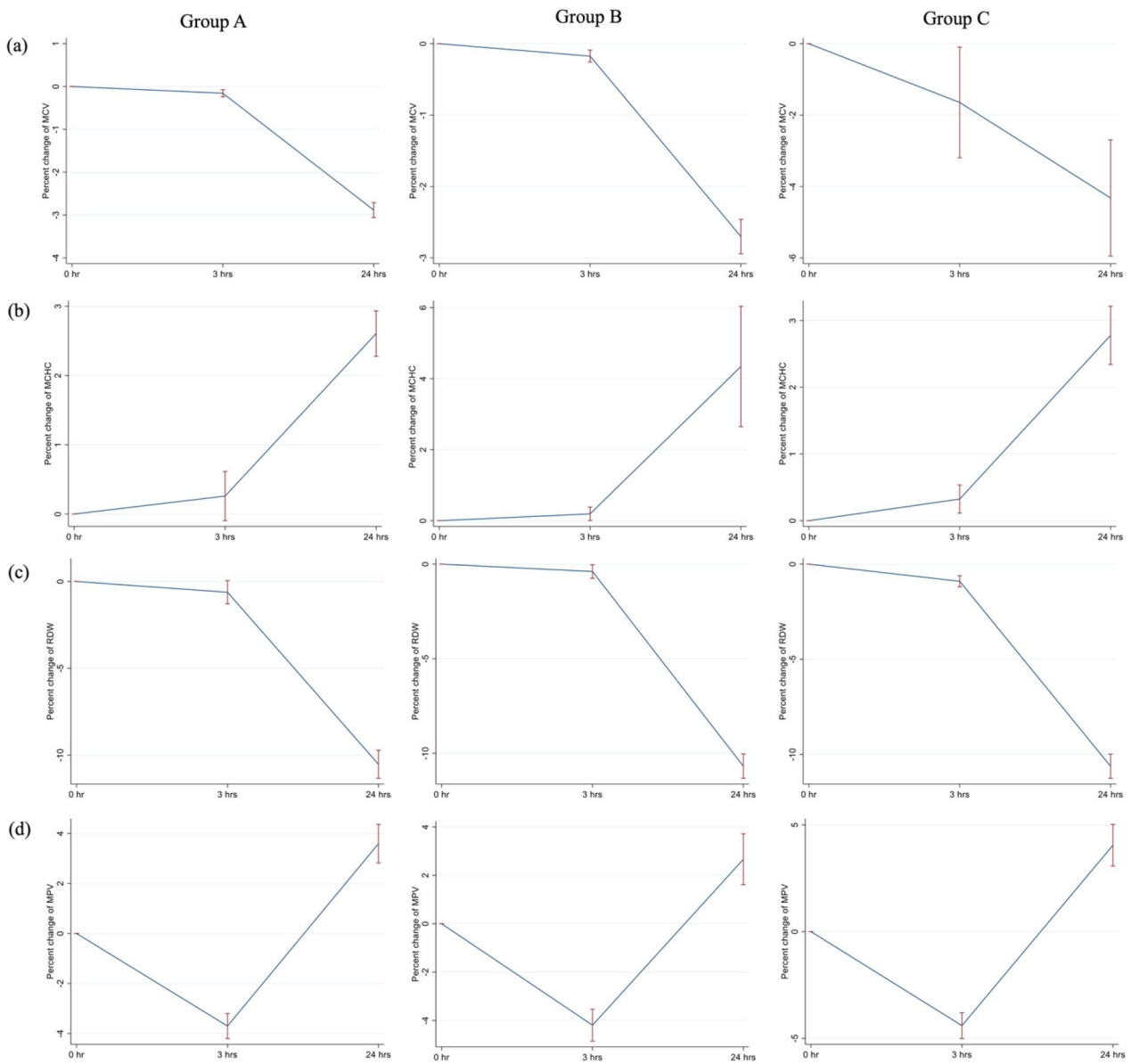


Figure 1. Percentage changes of (a) MCV, (b) MCHC, (c) RDW, and (d) MPV from three groups at 0, 3, and 24 hours. (A: vacuum K₂EDTA tubes, B: non-vacuum K₂EDTA tubes, C: non-vacuum K₂EDTA tubes with under-filled volumes)

MCV and RDW values increased within each group, while MCHC trended downward. Notably, MPV showed significant differences at 3 hours and 24 hours post-collection. Conversely, Hct, Hb, and MCH remained relatively stable, with no statistically significant changes in any group at 3 and 24 hours post-collection.

The hemolysis testing results, shown in **Table 2**, revealed statistically significant differences in hemolysis percentages among the three groups at 0 hours ($p = 0.012$), with group A exhibiting the highest hemolysis levels. Additionally, group C showed a statistically significant increase in

hemolysis from hour 3 onwards ($p = 0.028$ at 3 hours; $p = 0.009$ at 24 hours).

Discussion

The present study investigated the performance of K₂EDTA vacuum and non-vacuum blood collection tubes, including under-filled non-vacuum tubes, in automated CBC parameters to assess hemolysis occurrence at different time points.

At the time of blood collection, no significant differences were observed in CBC values among the vacuum tubes, non-vacuum tubes,

Table 2. Mean hemolysis was at 0 hours for three groups of K₂EDTA tubes (highlighted row), and the mean differences in hemolysis were at different time points.

	A	p-value within group A	B	p-value within group B	C	p-value within group C	p-value (compare three groups)
Hemolysis (%)	5.85±5.39	2.98±3.66	2.83±3.33	0.012*			
diffHemo (3 hr)	0.94±5.15	-1.1±3.74	-1.81±4.19	0.124	0.028*		0.053
diffHemo (24 hr)	-0.22±4.62	-0.46±4.08	-2.26±4.37	0.544	0.009*		0.155

A: vacuum K₂EDTA tubes, B: non-vacuum K₂EDTA tubes, C: non-vacuum KK₂EDTA tubes with under-filled volumes.

**p* < 0.05 is significant

and non-vacuum tubes with underfilled volumes. However, considerable parameter changes were observed within each tube type at 3- and 24-hour post-collection. Significant differences were found in the vacuum tubes (group A) at 3 hours post-collection, with WBC and MPV increasing while platelet count decreasing. This may be attributed to larger platelets, which are not counted as platelets by the analyzer, resulting in an apparent decrease in platelet count.^(20, 21) Additionally, a significant increase in MCV was observed in the non-vacuum tubes (group B), while RDW significantly changed in the non-vacuum tubes with underfilled volumes (group C). MPV showed significant changes across all three tube types from 3 hours onward.

At 24 hours post-collection, all three tube types showed significant changes in MCV, MCHC, and RDW, with RBC count significantly changing only in the vacuum tubes (group A). These findings suggest morphological and chemical alterations in red blood cells with prolonged storage at room temperature (RT).⁽²²⁾ Both vacuum and non-vacuum tubes, including underfilled tubes, can be used for hematological analysis within 3 hours post-collection. However, caution should be exercised regarding potential inaccuracies, as some parameters significantly changed after 3 hours, such as WBC and platelet count in vacuum tubes (group A), MCV in non-vacuum tubes (group B), and RDW in underfilled tubes (group C). MPV exhibited variability across all three tube types at 3- and 24-hour post-collection. This finding aligned with the results reported by Wu et al.⁽²³⁾, who conducted a systematic review and meta-analysis, highlighting that MPV was unstable for samples stored over time. In addition to the time interval between collection and analysis, temperature conditions must be considered to ensure reliable CBC analysis. If processing is delayed, samples should be stored at 4 °C to preserve stability.^(20, 23-25)

Interestingly, the highest hemolysis was observed in the vacuum tubes (group A) immediately after blood collection. This result was consistent with previous studies, indicating that blood collection using vacuum tubes was associated with a higher hemolysis rate than the aspiration

technique.^(15, 16) Hu et al.⁽⁸⁾ reported that vacuum tubes may cause increased hemolysis due to residual negative pressure, which exerts mechanical stress on red blood cells. *In vitro* hemolysis can produce unreliable laboratory results by releasing intracellular components, causing errors and misinterpretation.⁽¹⁹⁾ While vacuum blood collection tubes are widely used, the effect of hemolysis due to negative pressure should be considered, especially in patients suspected of hemolysis, as collection-induced hemolysis may interfere with the test results.

The disproportionate ratio of anticoagulant to blood for under-filled collection tubes can interfere with assays and lead to laboratory errors.⁽²⁶⁾ In this study, an initially disproportionate anticoagulant-to-blood ratio did not contribute to significant *in vitro* hemolysis. However, a significant increase in hemolysis was observed at 3- and 24-hour post-collection, suggesting that excessive EDTA may affect red blood cell stability with prolonged storage at RT.

This study encountered several limitations; firstly, the relatively small sample size may restrict the generalizability of the findings. Moreover, this study consisted exclusively of healthy individuals, which may not fully represent the variability observed in clinical populations. In addition, no information on the traits of thalassemia or hemoglobinopathies was collected, which could influence the increase in hemolysis at different times. Secondly, the analysis was conducted in a single laboratory without inter-laboratory validation, which may affect the reproducibility of results across various settings. Further studies involving larger and more diverse populations and multiple laboratories are recommended to validate and extend these findings.

Conclusion

This study evaluated the performance of K₂EDTA vacuum and non-vacuum blood collection tubes, including underfilled non-vacuum tubes, in automated CBC analysis and hemolysis testing over time. No significant differences in CBC values were observed among the three tube types immediately after blood collection. These

findings suggest that all three tube types are suitable for hematological analysis if processed within 3 hours. If analysis is delayed beyond the recommended time, samples should be stored at 4 °C for preservation. However, the risk of hemolysis in vacuum tubes should be carefully considered, particularly in individuals with suspected hemolysis. This study may provide a basis for the potential advancements in preanalytical quality control, particularly in the standardization of blood collection and handling protocols to minimize variability in routine hematological testing.

Acknowledgments

Phramongkutklo College of Medicine supported this work. We thank Ms. Supak Ukritchon from the Office of Research and Development, Phramongkutklo College of Medicine, for statistical analysis.

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