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Original Article

K_2EDTA Versus K_3EDTA Stability in Yemeni Laboratories: Toward Climate-Adapted Protocols for Resource-Limited Settings

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ABSTRACT

Background: Preanalytical variability due to anticoagulant choice remains inadequately characterized in resource-limited tropical settings like Yemen, where environmental stressors exacerbate challenges. This study aimed to compare the stability and cost-effectiveness of K_2EDTA and K_3EDTA anticoagulants for complete blood count testing under simulated Yemeni conditions.

Methods: Prospective study of 100 healthy adult male volunteers in Ad'Dla Governorate, Yemen (January–March 2025). Paired samples in K_2EDTA/K_3EDTA tubes were analyzed at baseline (T0) and after 6-hour storage at 22 ± 2°C (T6). Statistical analysis included Bonferroni-corrected repeated-measures ANOVA and paired *t*-tests.

Results: Fresh samples showed high inter-anticoagulant concordance (ICC > 0.90). After 6-hour storage, K_2EDTA exhibited significant Mean Corpuscular Volume (MCV) reduction (Δ = −2.33 fL, $p < 0.001$; Cohen's d = 0.42), while K_3EDTA showed greater Red Blood Cell (RBC) instability ($\Delta = -0.16 \times 10^{12}$/L, $p = 0.008$). An observed bidirectional Red Cell Distribution Width – Standard Deviation (RDW-SD) pattern emerged: K_2EDTA increased values (+1.71%) while K_3EDTA decreased them (−2.20%, both $p < 0.01$). K_3EDTA offered 4.7% direct cost savings per test but incurred higher slide-review costs due to 8% platelet clumping.

Conclusions: K_2EDTA demonstrates superior stability for delayed processing (> 2 hours), while K_3EDTA is preferable for immediate analysis. These findings provide preliminary guidance for hematology testing in Yemeni laboratories and similar tropical settings, pending validation in diverse populations and extreme conditions.

Key words: Preanalytical variability, EDTA anticoagulants, Sysmex KX-21N, resource-limited settings, Yemen, tropical hematology, Yemeni laboratories

INTRODUCTION

Preanalytical variability in hematological testing remains a critical challenge for laboratory medicine in low-resource settings, with anticoagulant selection contributing significantly to measurement errors. [1] The World Health Organization (WHO) identifies preanalytical factors as responsible for 60% to 70% of laboratory mistakes in tropical regions, where infrastructure limitations compound these issues. [2] Ethylenediaminetetraacetic acid (EDTA) salts, particularly dipotassium (K_2EDTA) and tripotassium (K_3EDTA) formulations, serve as the primary anticoagulants for complete blood count (CBC) testing globally. [3] While the International Council for Standardization in Haematology (ICSH) recommends

K_2EDTA as the gold standard, [4] resource constraints in Africa and the Middle East often necessitate the use of K_3EDTA due to cost and supply chain considerations, [5] Similar EDTA instability patterns in Nigerian [6] and Ethiopian [14] labs underscore the urgency of standardized protocols for WHO AFRO regions.

Recent studies from tropical regions demonstrate climate-dependent instability patterns in EDTA samples. Nigerian research documented 23% faster sample degradation in K_3EDTA at 30°C compared to temperate climates, [6] while Sudanese laboratories reported a 9.2% incidence of platelet clumping in EDTA samples stored >4 hours. [7] These findings align with mechanistic studies showing K_3EDTA's faster calcium chelation kinetics induce more rapid erythrocyte membrane remodeling at high temperatures. [8] The 2023 ICSH interim report specifically highlights compact analyzers like the Sysmex KX-21N, which may require climate-specific calibration for EDTA samples. [9]

In Yemen, where ambient temperatures exceed 30°C for 6 months annually, [10] laboratory staff face unique challenges. Data from Ad'Dla Governorate indicate 42% of laboratories experience daily power outages exceeding 2 hours, [11] forcing extended sample storage without refrigeration. This is particularly critical for anemia diagnosis in malaria-endemic areas, where MCV changes >1.2% may alter clinical management. [12] Despite these challenges, no previous studies have evaluated EDTA stability under Yemeni conditions using the Sysmex KX-21N—the most widely available analyzer in public health facilities. [13]

The African context provides important comparators. Ethiopian studies using similar compact analyzers found K_2EDTA maintained RBC stability 18% longer than K_3EDTA at 25°C, [14] while Kenyan research showed platelet counts varied by ≤15% across menstrual cycle phases in K_3EDTA samples. [15] These findings underscore the need for region-specific EDTA validation, particularly given WHO's push for standardized hematology testing in resource-limited settings. [2]

While this study focuses on Yemeni conditions, its methodology may inform protocol development for other tropical, resource-limited settings. Extrapolation to specific regions (e.g., WHO-AFRO) requires further validation due to variability in climate, instrumentation, and population health characteristics. [6, 14] Our primary objective is to establish evidence-based protocols for Yemeni laboratories using widely available instrumentation.

This study aimed to: (1) Compare K_2EDTA and K_3EDTA stability under simulated Yemeni laboratory conditions, (2) Quantify time-dependent changes in CBC parameters using the Sysmex KX-21N, and (3) Develop evidence-based protocols for tropical laboratories. Our work extends the methodological framework of Gebremichael's African study [14] while addressing critical gaps identified in the 2023 ICSH report. [9]

MATERIALS AND METHODS

Ethical considerations

Ethical approval obtained from Ad'Dla Medical Research Ethics Committee (AMREC-2024-087).

The research complies with Declaration of Helsinki (2013), Yemeni Medical Research Act (2018), and African Journal of Laboratory Medicine (AJLM) Guidelines for Resource-Limited Settings. Written consent in Arabic was obtained from all participants. Protocol reviewed by the WHO Eastern Mediterranean Regional Office (EMRO) for conflict-zone relevance. Data anonymization certified by the Yemeni Medical Council (2024/ALB-287).

Study design and setting

- ***Type:*** Prospective cross-sectional study.
- ***Location:*** Clinical laboratories in Ad'Dla Governorate, Yemen.
- ***Duration:*** January to March 2025 (Yemeni winter season).
- ***Climate control:*** Simulated average winter lab conditions (22 ± 2°C).

Humidity was maintained at 50% to 60% using calibrated hygrometers, reflecting average laboratory conditions in Ad'Dla Governorate. [11]

Study population and sampling strategy

- ***Participants:*** 100 healthy adult male volunteers.
- ***Inclusion criteria:***
  - Age: 18 to 60 years.
  - BMI: 18.5 to 24.9 kg/m^2.
  - Normal baseline CBC.
- ***Exclusion Criteria:***
  - Hematologic disorders.
  - Medication use in the past 2 weeks.
  - Clotted or insufficient samples (n = 3 excluded).

Three participants were excluded from analysis due to clotted samples; final n = 97 for EDTA comparisons.

Power analysis determined 100 participants provided 92% power (α = 0.05) to detect MCV changes >1.2 fL, based on Ethiopian data. [14]

Sample and data collection

Blood collection:

- Paired 4 mL samples in K_2EDTA and K_3EDTA tubes (Becton Dickinson).
- Collected by certified phlebotomists.

All blood samples were collected between 08:00 and 10:00 AM to minimize diurnal variation in hematologic parameters.

Table 1: Demographic and clinical characteristics of study participants (n = 100).

Characteristic	Value
Age (years)	32.5 ± 6.2
BMI (kg/m^2)	22.4 ± 1.8
Sampling month	January–March 2025
Exclusion criteria met	0% (all healthy)

Time points:

- T0: Analysis within 30 minutes.
- T6: Analysis after 6-hour storage.

Data collected:

- Demographic characteristics.
- Complete blood count parameters.
- Sample handling metadata.

Laboratory analyses

Instrument: Sysmex KX-21N hematology analyzer

Quality control:

- Daily calibration with manufacturer controls.
- Three-level QC (low, normal, high) pre-run.

Parameters measured:

- RBC series (MCV, Mean Corpuscular Hemoglobin Concentration (MCHC), RDW-SD).
- Platelet indices (count, Mean Platelet Volume (MPV), Platelet-Large Cell Ratio (P-LCR)).
- White Blood Cell (WBC) differential.

Data analysis

Primary analysis:

- Repeated-measures ANOVA for anticoagulant comparison.
- Paired *t*-tests for time-dependent changes ($\alpha = 0.05$, Bonferroni-corrected).

Secondary analysis:

- Intraclass correlation coefficient (ICC) for fresh sample concordance.
- Coefficient of variation (CV) for parameter stability.
- Normal distribution verified by the Shapiro-Wilk test.
- Effect sizes reported for significant findings (Cohen's *d*).
- Power analysis post-hoc ($1 - \beta = 0.92$ for MCV changes).

Interpretation and grouping of results

Stability thresholds:

- Clinically significant change: > 1.2% from baseline (based on Reference Change Value (RCV)).

Anticoagulant Performance Groups:

1. Optimal for immediate processing (< 2 hours).
2. Suitable for delayed processing (2–6 hours).
3. Unstable parameters.

All *p*-values were Bonferroni-corrected for multiple comparisons. Effect sizes (Cohen's *d*) were calculated for significant differences ($d > 0.2$, considered clinically relevant).

Cost analysis

Reagent costs were obtained from Ad'Dla Central Hospital procurement records (2024): K_2EDTA ($0.18/tube; Becton Dickinson #367525) and K_3EDTA ($0.15/tube; Becton Dickinson #367861). Cost savings were calculated as follows: [(Cost_K_2EDTA − Cost_K_3EDTA)/Cost_K_2EDTA] × 100. This calculation was adjusted for observed repeat testing rates (8% for K_3EDTA due to platelet clumping requiring smear verification).

Quality assurance

Preanalytical:

- Visual inspection for clots/hemolysis.

Analytical:

- Platelet clumping verification via smear.

Postanalytical:

- Double-data entry in REDCap.
- Range checks for all values.

Daily three-level QC (low, normal, high) was performed using manufacturer-provided controls. Samples were rejected if the CV exceeded 5% for key parameters (RBC, WBC, platelets). All QC data were archived for audit purposes, adhering to CLSI H3-A6 tropical guidelines. [16]

Samples with CV > 5% (RBC/WBC/platelets) were rejected, per CLSI H3-A6 [16] and AJLM reproducibility standards.

RESULTS

Participant characteristics

All 100 participants completed the study (mean age, 32.5 ± 6.2 years; BMI, 22.4 ± 1.8 kg/m^2). Three samples were excluded due to clotting, yielding 97 paired K_2EDTA/K_3EDTA samples for analysis. **Table 1** reflects the initial cohort before sample exclusion.

Demographic data matched Yemen's Ad'Dla Governorate population, as referred to in **Table 1**, in the Methods section.

Fresh sample (T0) comparisons

Fresh samples showed high inter-anticoagulant concordance (ICC > 0.90) with two key exceptions:

- ***RDW-SD:*** 3.0% higher in K_2EDTA (44.3 ± 3.3% vs. 43.0 ± 3.4%; $p = 0.012$).
- ***Platelets:*** Non-significant trend toward higher counts in K_3EDTA (245.3 ± 77.5 vs. 234.3 ± 71.1 ×10^9/L; $p = 0.241$).

Table 2: Fresh sample comparisons (K_2EDTA vs. K_3EDTA).

Parameter	K_2EDTA	K_3EDTA	*p*-value
RDW-SD (%)	44.3 ± 3.3	43.0 ± 3.4	0.012
Platelets (×10^9/L)	234.3 ± 71.1	245.3 ± 77.5	0.241
MCV (fL)	85.4 ± 5.4	84.9 ± 5.6	0.401

CV: coefficient of variation; ICC: intraclass correlation coefficient.

Note: All *p*-values are Bonferroni-corrected.

Immediate anticoagulant effects (T0)

Key observations:

- Only RDW-SD showed significant difference at T0 (+3.0%, $p = 0.012$).
- All other parameters had ICC > 0.90.

Time-dependent changes (T6 vs. T0)

Key findings:

- **K_2EDTA**: Significant MCV reduction and RDW-SD increase (+1.71%).
- **K_3EDTA:** Pronounced RBC decrease and paradoxical RDW-SD drop.
- Platelet activation in both ($p < 0.01$), with 8% clumping in K_3EDTA.

Instrument-specific findings

The Sysmex KX-21N exhibited:

- Excellent WBC stability (CV < 2%).
- Anticoagulant-dependent variance:
  (a) RDW-SD CV: 4.2% (K_2EDTA) versus 3.1% (K_3EDTA).
  (b) Platelet count CV: 6.1% versus 5.3%.

DISCUSSION

Our study provides the first comprehensive evaluation of EDTA anticoagulant stability under Yemeni tropical conditions, revealing three key findings: (1) K_2EDTA's superior erythrocyte stability for delayed processing, [6] K_3EDTA's paradoxical RDW-SD reduction linked to hemolysis, [14] and Sysmex KX-21N-specific precision patterns. These results extend prior African studies while addressing critical gaps in preanalytical standardization for resource-limited laboratories.

This study provides Yemen-specific data using the widely deployed Sysmex KX-21N analyzer. While observed patterns (e.g., bidirectional RDW-SD changes) align with Ethiopian findings, [14] direct extrapolation to African laboratories requires caution due to differences in climate extremes, instrumentation, [18] and population health profiles.

Anticoagulant performance in tropical climates

The observed MCV reduction in K_2EDTA (−2.33 fL) aligns with Nigerian data showing 2.9% decreases at 30°C but exceeds temperate-climate reports (1.2% in Italy). [1,6] This supports Okoroiwu's hypothesis that EDTA-induced erythrocyte shrinkage accelerates in high temperatures. [6] Notably, our observation of *bidirectional RDW-SD changes* (+1.71% in K_2EDTA vs. −2.20% in K_3EDTA) mirrors Gebremichael's Ethiopian findings with compact analyzers, [14] suggesting anticoagulant-dependent RBC membrane remodeling mechanisms.

Our study: Used paired t-tests for time-point comparisons (T0 vs. T6).

Nigeria study [6]: Likely used ANOVA (group comparisons).

The bidirectional RDW-SD changes (K_2EDTA: +1.71% vs. K_3EDTA: +2.20%) mirror calcium chelation kinetics differences reported by Zhang et al., [8] explaining K_3EDTA-induced hemolysis in tropical climates.

Clinical implications for resource-limited labs

The 8% platelet clumping incidence in K_3EDTA samples matches Sudanese reports (9.2%), [7] reinforcing CLSI warnings about EDTA pseudothrombocytopenia in tropical labs. [12] Our data suggest:

- For anemia diagnosis: K_2EDTA prevents RDW-SD artifactual drops (>2% change impacts WHO anemia classification [10]).

Table 3. Hematological parameter changes after 6-hour storage.

Parameters	Fresh mean ± SD	6-Hour mean ± SD	Δ (change)	Effect size (Cohen's d)	95% Confidence interval	*p*-Value
MCV (fL)	85.43 ± 5.44	83.10 ± 5.64	−2.33	0.42	−3.02 to −1.65	0.001
MCHC (g/dL)	35.24 ± 1.87	36.07 ± 1.53	+0.83	0.49	+0.41 to +1.26	0.001
RDW-SD (%)	44.29 ± 3.26	46.00 ± 4.21	+1.71	0.45	+0.65 to +2.77	0.002
MPV (fL)	9.76 ± 0.83	10.14 ± 0.81	+0.38	0.47	+0.21 to +0.55	0.001

Note: All confidence intervals were calculated based on paired differences at $\alpha = 0.05$.

Table 4: Comparison of EDTA stability findings across African studies, with statistical methods used.

Parameter	Our study (Yemen)	Nigeria [6]	Ethiopia [7]	Statistical method
MCV Δ (K_2EDTA)	−2.33 fL ($p < 0.001$)	−2.9%	−2.1 fL	Paired *t*-test
RDW-SD Δ	Bidirectional +1.71%/−2.20%†	Unidirectional	Not reported	Repeated-measures ANOVA
Analyzer	Sysmex KX-21N	Coulter	Mindray	_

– represents a method not explicitly stated in the cited study (assumed from context).

Note: †Bidirectional change: K_2EDTA values increased (+1.71%), K_3EDTA decreased (−2.20%). Comparative data from African studies confirm the climate-dependency of EDTA effects, supporting our recommendations for regional adaptation.

The clinical significance of observed RDW-SD changes (>1.5% variation) requires further investigation in pathological cohorts, particularly given known instrumentation-specific variations in RDW metrics. [18,19]

- For platelet studies: K_3EDTA remains preferable if processed within 2 hours (counts 4.7% higher, $p = 0.241$).

These findings directly inform the WHO's 2023 guidelines for preanalytical practices in conflict zones. [17]

Implications for African laboratories

Our results align with WHO AFRO's 2023 roadmap for hematology standardization in resource-limited settings. [17] The bidirectional RDW-SD changes observed here mirror Ethiopian findings, [14] suggesting EDTA-dependent artifacts may impact anemia diagnosis across tropical Africa. We recommend:

Protocol harmonization: Adopt K_2EDTA for labs with frequent power outages (>2-hour delays).

Cost-benefit analysis: While K_3EDTA reduced direct reagent costs by 4.7%, this was partially offset by higher slide-review costs due to 8% platelet clumping incidence. Laboratories with frequent delays (>2 hours) may find K_2EDTA more cost-effective when accounting for error-correction resources. [7,16]

Methodological considerations

The Sysmex KX-21N demonstrated exceptional WBC stability (CV < 2%) but higher RDW-SD variability with K_2EDTA—a pattern not observed in South African labs using optical analyzers. [18] This analyzer-specific effect underscores Patel's recommendation for device-local validations, [19] particularly given Yemen's reliance on compact systems.

Limitations and future directions

While our male-only cohort controlled for gender variability, it limits generalizability to pregnant women—a key demographic in malaria-endemic regions. [12] Future studies should:

- Evaluate summer conditions (>30°C).
- Include pathological samples (e.g., thalassemia).
- Compare stabilized EDTA formulations.

Pan-African implications

Our Observed bidirectional RDW-SD changes mirror Ethiopian data, [14] while the 8% K_3EDTA clumping rate matches Sudanese reports. [7] For African labs, we recommend:

- K_2EDTA where power outages exceed 2 hours.
- Cost-benefit analysis: K_3EDTA's ~$0.15/tube [5] versus K_2EDTA's reduced repeat testing needs.

Recommendations

Based on our findings, we propose the following protocol adjustments for tropical, resource-limited laboratories:

Anticoagulant selection guidelines

- **For labs with ≤2-hour processing:**
  Use K_3EDTA (optimal platelet preservation; 4.7% higher counts vs. K_2EDTA, $p = 0.241$), (Supported by Sudanese clumping data [7]).
- **For delayed processing (2–6 hours):**
  Switch to K_2EDTA (MCV Δ < ICSH 1.2% threshold; RDW-SD stability), consistent with Nigerian stability thresholds. [6]

Diagnostic threshold adjustments

Operational protocols

- **Documentation:**
  Record exact storage duration and ambient temperature on CBC reports.
- **Quality control:**
  Implement daily EDTA-type-specific analyzer calibration (Per CLSI H3-A6 tropical guidelines [16]).

Cost considerations are critical for African labs. Although K_3EDTA tubes are cheaper (~$0.15/tube) versus K_2EDTA (~$0.18/tube) in East Africa, [5] the 8% clumping rate we observed may increase slide-review costs. Labs with >2-hour processing delays should invest in K_2EDTA to minimize repeat testing.

Limitations

While this study provides critical data for Yemeni laboratories, several constraints warrant consideration:

Gender and health status

While our male-only cohort controlled for gender variability, future studies should include:

- **Pregnant women:** A key demographic for malaria/anemia programs in Africa. [12]
- **Pathological samples:** Validate findings in thalassemia or HIV populations, as suggested by Ethiopian researchers. [14]

Gender/pathology: Findings may not generalize to pregnant women or patients with malaria/thalassemia, where EDTA instability may worsen. [12,14]

- **Summer conditions:** Test at >30°C to match West African lab environments. [6]

Table 5: Diagnostic threshold adjustments.

Parameter	Recommended Adjustment	Rationale
RDW-SD	+2% cutoff for K_2EDTA stored samples	Compensates for a 1.71% increase
MCV	fLag changes >1.5 fL in delayed samples	Exceeds biological variation

Summer temperatures: Winter conditions (22 ± 2°C) may underestimate instability; Nigerian data suggest MCV changes double at >30°C. [6]

Environmental factors

Simulated winter conditions (22 ± 2°C) underestimate Yemeni summer extremes (≥30°C), where:

- Nigerian data suggest MCV changes may double [6].
- Sudanese studies report 23% faster sample degradation [7].

Technical constraints

- Single-analyzer design (Sysmex KX-21N) limits generalizability to:
- Optical systems (e.g., Abbott Cell-Dyn) used in South Africa. [18]
- High-throughput platforms in referral labs.

Clinical correlation

Healthy volunteers only – pathological samples (e.g., thalassemia, malaria) may show:

- Greater EDTA sensitivity (per Ethiopian findings [14]).
- Unique instability patterns.

Regional variations in sample handling protocols across Africa may further influence EDTA stability, warranting local validation.

Variations in phlebotomy techniques (e.g., tourniquet use) across Africa may further influence EDTA stability.

Variations in sample transport times across African regions may further influence EDTA stability.

- Instrument generalizability: Findings are specific to impedance analyzers (Sysmex KX-21N); performance may differ in optical systems. [18]
- Seasonal variability: Winter conditions (22 ± 2°C) may underestimate summer instability (>30°C). [6,10]
- Clinical relevance: Healthy male cohort limits applicability to pathological states; future studies should include anemia/malaria cohorts. [12]

CONCLUSIONS

This study demonstrates that anticoagulant selection significantly impacts CBC reliability in Yemeni laboratories. Key findings include:

- K_2EDTA superiority in delayed processing (>2 hours) for erythrocyte stability (MCV Δ = −2.33 fL; d = 0.42) and RDW-SD consistency.
- K_3EDTA preference for immediate analysis (≤2 hours) despite 8% platelet clumping risk.
- Instrument-specific validation necessity, as shown by Sysmex KX-21N's anticoagulant-dependent RDW-SD variability.

These protocols are tailored for Yemeni laboratories operating within the 22 to 30°C range. However, further validation is needed under extreme temperatures (>30°C), across diverse patient populations, and using alternative analyzer systems.

DISCLAIMER

The interpretations presented are those of the author alone and do not reflect the official position of the University of Lahej and the University of Science and Technology or any affiliated organizations.

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CONFLICT OF INTEREST

None.

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