

Original Article

EXTENDED IN-USE STABILITY OF KREBS VON DEN LUNGEN-6 (KL-6) REAGENTS, QUALITY CONTROLS, AND CALIBRATORS UNDER REFRIGERATED CONDITIONS

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ABSTRACT

Reliable diagnostic testing depends on the stability of assay components, including reagents, quality controls (QCs), and calibrators. For the Nanopia KL-6 assay used in interstitial lung disease (ILD) assessment, the manufacturer states that in-use stability is limited to 1 month for reagents and QCs and approximately 2.5 months for calibrators at 2–8°C. This study evaluated the extended in-use stability of Nanopia KL-6 reagents, QCs, and calibrators for up to four months under refrigerated conditions. Extended stability testing was performed at seven predefined time points (T0–T6) over 132 days. Reagent stability was assessed using in-use QC materials at two concentration levels. QC stability was evaluated by aliquoting and testing stored QC material over time. Calibrators stability was assessed across four concentration levels using bias analyses. Reagents and QCs demonstrated acceptable analytical stability for up to 132 days, with increasing bias observed beyond Day 90. All calibrator levels remained stable for up to 132 days with minimal bias (<10%). Nanopia KL-6 reagents, QCs and all calibrator levels remain stable for up to 132 days when stored at 2–8°C, provided appropriate quality monitoring is implemented.

KEYWORDS: KL-6, Krebs von den Lungen-6, Nanopia, stability, extended

INTRODUCTION

Ensuring the quality and stability of diagnostic reagents and materials is fundamental to achieving accurate laboratory results and supporting reliable clinical decision-making in diagnostic medicine (1). Degradation of samples or reagents during pre-analytical processes, including storage, handling, and transportation, can significantly compromise analytical accuracy and may lead to erroneous results and clinical misinterpretation (2). This issue is particularly critical for biomarkers such as Krebs von den Lungen-6 (KL-6), where analytical variability may directly influence disease monitoring, prognosis, and therapeutic decision-making in interstitial lung diseases (ILD) (3,4).

KL-6 is a high-molecular-weight mucin-like glycoprotein classified as human MUC1 and is predominantly expressed on regenerating type II alveolar epithelial cells in the lung (3). Elevated serum KL-6 concentrations have been widely recognised as a sensitive biomarker for the diagnosis and prognostic assessment of ILD, including connective tissue disease-associated ILD (4). Measurement of KL-6 is routinely performed using immunoassay-based platforms such as latex agglutination assays and enzyme-linked immunosorbent assays (ELISA), which rely heavily on reagent and calibrator integrity for accurate quantification (5).

Despite extensive literature supporting the biological and clinical relevance of KL-6, limited data are available regarding the long-term stability of commercial KL-6 assay components under extended in-use conditions (1). In routine clinical laboratories, reagents, calibrators, and QC materials are often stored and used over long periods before full consumption, making stability verification essential to minimise analytical variability (6). Stability studies, including shelf-life and in-use evaluations, assess product performance over time under defined storage conditions, ensuring consistency and reliability (1).

Regulatory and professional guidelines recommend long-term stability testing of in vitro diagnostic reagents for at least three to six months when stored under refrigerated conditions, particularly when extended use is anticipated in clinical laboratories (1). Previous studies have demonstrated that many biomarkers retain acceptable stability beyond manufacturer-stated limits when appropriately stored, highlighting the importance of laboratory-based validation studies (6,7).

This study evaluated the extended in-use stability of the Nanopia KL-6 reagent, quality control materials (Level 1 and Level 2), and calibrators (Levels 1–4) stored at 2–8°C for up to 132 days. The objective was to generate evidence-based storage timelines that maintain analytical performance and support consistent and reliable clinical application of KL-6 testing in routine diagnostic laboratories (6).

MATERIALS AND METHODS

An isochronous stability design was employed. Nanopia KL-6 reagent, quality control materials, and calibrators (500–5000 U/mL) were stored according to the manufacturer's instructions and analysed at seven time points from Day 1 (T0) to Day 132 (T6). KL-6 concentrations were measured using a chemistry analyser, Beckman Coulter AU480 (Infinitive Medic Sdn Bhd), which was located at Special Protein Unit, Specialised Diagnostic Centre, Institutes for Medical Research, Jalan Pahang, Kuala Lumpur, Malaysia. Each sample was analysed in duplicate, and the mean value was calculated. Bias

(%) was calculated relative to T0. Percentage bias was calculated relative to the baseline value (T0) using the following equation: $\text{Bias (\%)} = [(C_t - C_{t0}) / C_{t0}] \times 100$, where C_t represents the mean KL-6 concentration at each time point and C_{t0} represents the mean concentration at baseline. An allowable bias criterion of $\pm 15\%$ was applied in accordance with published analytical performance recommendations (8) and the manufacturer's allowable bias limit.

Reagent Stability

A new bottle of Nanopia KL-6 reagent was stored continuously at 2–8°C and used beyond the manufacturer's recommended in-use period to evaluate its extended stability. The reagent bottle was opened and placed on the analyser at the start of the study and remained in use throughout the testing period of up to 132 days. To assess reagent stability, on-board QC materials (both Level 1 and Level 2) were used as test samples. These QC materials were freshly prepared, verified to be within their respective stability periods, and stored under proper conditions (2–8°C) throughout the study. QC testing was performed at seven defined time points (T0 to T6), where T0 represented the initial day the reagent bottle was opened and T1 to T6 represented subsequent time points up to 132 days from opening. At each time point, the stored reagent was used to analyse QC materials, and the results were compared to baseline values obtained at T0. The aim was to observe any drift or degradation in reagent performance over time. All QC testing results were collected and compiled for analysis. Data analysis focused on detecting significant deviations in QC results attributable to extended reagent storage, thereby assessing its functional stability over the 132-day period.

Quality Control Stability

Nanopia KL-6 Quality Control (QC) material was stored at 2–8°C until the stability study began. To simulate real sample handling, the QC material was treated as patient samples. The QC solution was aliquoted into seven equal portions of 55 μL each, using secondary tubes. At the initial time point (T0), one aliquot was immediately analysed to establish the baseline measurement. The remaining six aliquots were stored under the same 2–8°C refrigerated condition and were designated for subsequent testing at later time points. The stored QC aliquots were tested at six additional time points (T1 to T6), according to a predefined schedule up to 132 days from the opening. All results collected at each time point were compiled and analysed to evaluate any time-dependent changes in QC performance, with the goal of determining the material's stability and suitability for use beyond the manufacturer's specified duration.

Calibrator Stability

The KL-6 calibrator was stored at 2–8°C after opening and evaluated for extended in-use stability over up to 132 days. The calibrator, consisting of four concentration levels (Level 1 to Level 4), was treated as the test sample in this study. At the start of the study (T0), each calibrator level was tested using an on-board reagent that was within its validated in-use period. This initial measurement served as the baseline for stability comparison. Each calibrator level was aliquoted into secondary tubes and stored at 2–8°C. At defined time points (T1 to T6), stored calibrator aliquots were retrieved and tested again using freshly prepared quality controls and the same on-board reagent to assess stability. The test time points included T0 as a baseline measurement immediately after calibrator preparation and T1 to T6 as subsequent stability checkpoints up to day 132. Changes in the calibrator values were monitored over time, using T0 results as the reference and compared them with the in-use stability of Nanopia KL-6 calibrators.

RESULTS

Reagent Stability

Table 1. Bias (%) of Level 1 and Level 2 quality control concentrations of KL-6 relative to T0

Time point	L1 (U/mL)	L1 Bias (%)	L2 (U/mL)	L2 Bias (%)
T0	366.6	0.00	953.9	0.00
T1	369.9	0.90	944.5	-0.99
T2	367.4	0.22	957.5	0.38
T3	379.1	3.41	965.7	1.24
T4	374.9	2.26	961.4	0.79
T5	374.1	2.05	973.5	2.05
T6	380.8	3.87	979.4	2.67

Note: T0 = Day 1; T1 = Day 30; T2 = Day 60; T3 = Day 90; T4 = Day 108; T5 = Day 120; T6 = Day 132

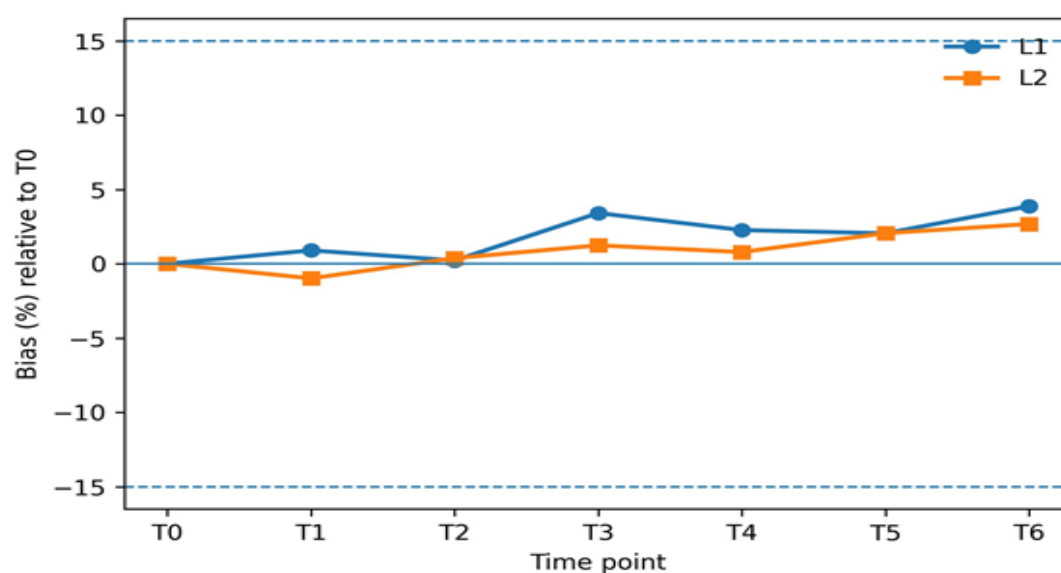


Figure 1. Bias (%) of Level 1 and Level 2 quality control concentrations of KL-6 relative to T0 across time points (T0–T6). Note: T0 = Day 1; T1 = Day 30; T2 = Day 60; T3 = Day 90; T4 = Day 108; T5 = Day 120; T6 = Day 132

Bias was assessed by determining the percentage bias at each time point (T1–T6) relative to the baseline measurement (T0), which served as the target value. The calculated biases were evaluated against the manufacturer’s allowable bias specification of $\pm 15\%$. As summarised in Table 1, the observed bias values for both concentration levels remained small throughout the study period. For L1,

bias values ranged from 0.22% to 3.87%, while for L2, bias values ranged from -0.99% to 2.67%. Figure 1 confirms that all bias values for both L1 and L2 remained within the predefined allowable limits of $\pm 15\%$ from T1 to T6. These findings indicate that analytical accuracy was maintained throughout the study duration when T0 was used as the reference. Overall, the bias acceptance criterion was fulfilled for both concentration levels in accordance with the manufacturer's performance claims.

QC Stability

Table 2. Bias (%) of Level 1 and Level 2 quality control concentrations of KL-6 relative to T0

Time point	L1 (U/mL)	L1 Bias (%)	L2 (U/mL)	L2 Bias (%)
T0	366.6	0.00	953.9	0.00
T1	369.9	0.90	944.5	-0.99
T2	368.7	0.57	956.7	0.29
T3	360.3	-1.72	955.9	0.21
T4	367.8	0.33	984.4	3.20
T5	399.2	8.89	957.5	0.38
T6	401.3	9.47	1029.9	7.97

Note: T0 = Day 1; T1 = Day 30; T2 = Day 60; T3 = Day 90; T4 = Day 108; T5 = Day 120; T6 = Day 132

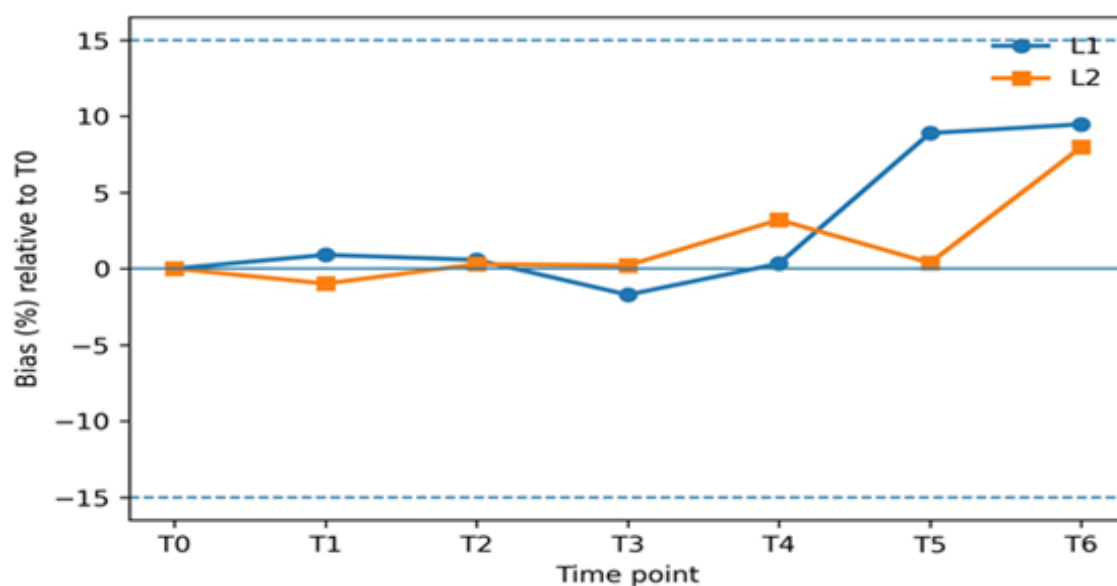


Figure 2. Bias (%) of Level 1 and Level 2 quality control concentrations of KL-6 relative to T0 across time points (T0–T6). Note: T0 = Day 1; T1 = Day 30; T2 = Day 60; T3 = Day 90; T4 = Day 108; T5 = Day 120; T6 = Day 132

As shown in Table 2, the Level 1 and Level 2 quality control materials demonstrated acceptable stability throughout the 132-day study period. Bias values for both QC levels remained within the pre-defined acceptance limit of $\pm 15\%$ relative to T0. Level 1 bias ranged from -1.72% to 9.47% , while Level 2 bias ranged from -0.99% to 7.97% . The highest bias for both QC levels was observed at T6 (Day 132), with values of 9.47% for Level 1 and 7.97% for Level 2. Overall, both QC levels remained analytically stable throughout the study period. Figure 2 demonstrates that QC materials exhibited minimal bias at baseline (T0) and maintained stable performance from T1 through T4. At T5, a slightly positive bias was observed, particularly in the low QC level. At T6, a mild positive bias was noted across both QC levels. Increased variability was observed at later time points, indicating declining stability beyond approximately 3 months of storage at $2-8^{\circ}\text{C}$. Overall, both concentration levels met the bias acceptance criterion, consistent with the manufacturer's performance claims.

Calibrator Stability

Table 3. Bias (%) of calibrator concentrations of KL-6 relative to T0

Time point	Level 1 (U/mL)	Level 2 (U/mL)	Level 3 (U/mL)	Level 4 (U/mL)	Level 1 Bias (%)	Level 2 Bias (%)	Level 3 Bias (%)	Level 4 Bias (%)
T0	537.50	1074.76	3236.82	5264.34	0	0	0	0
T1	504.14	1027.95	3097.68	5010.36	-6.21	-4.36	-4.30	-4.82
T2	492.54	997.28	2960.38	4969.00	-8.36	-7.21	-8.54	-5.61
T3	498.34	999.61	2949.08	5010.36	-7.28	-6.99	-8.89	-4.82
T4	483.82	999.61	2977.62	4939.32	-9.99	-6.99	-8.01	-6.17
T5	498.76	1011.66	3003.38	5014.80	-7.21	-5.87	-7.21	-4.74
T6	489.22	997.66	2969.59	4915.72	-8.98	-7.17	-8.26	-6.62

Note: T0 = Day 1; T1 = Day 30; T2 = Day 60; T3 = Day 90; T4 = Day 108; T5 = Day 120; T6 = Day 132

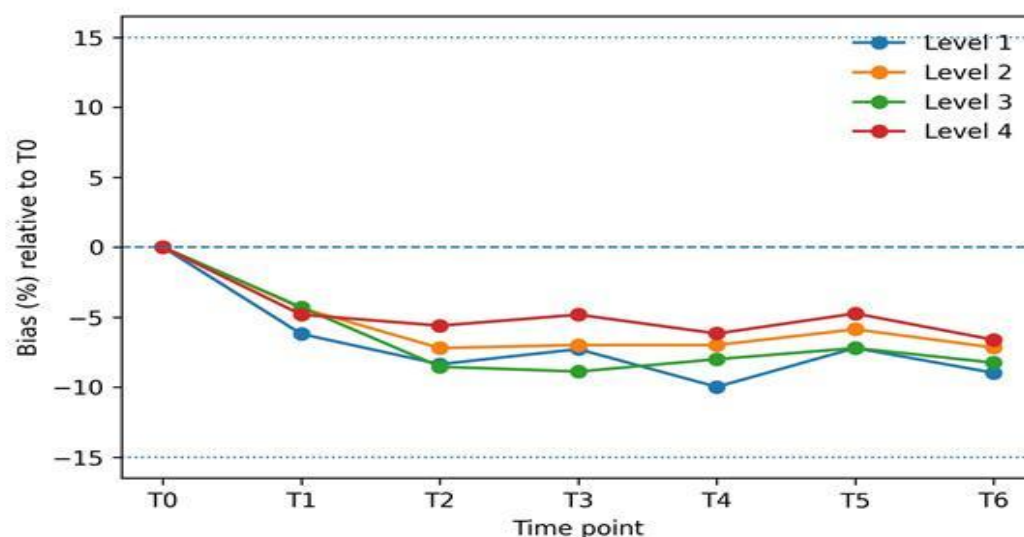


Figure 3. Bias (%) of Level 1, Level 2, Level 3 and Level 4 calibrator concentrations of KL-6 relative to T0 across time points (T0–T6). Note: T0 = Day 1; T1 = Day 30; T2 = Day 60; T3 = Day 90; T4 = Day 108; T5 = Day 120; T6 = Day 132

As summarised in Table 3, all four concentration levels showed bias values within the predefined acceptance limits throughout the study period. No progressive or excessive deviation from the target value was observed across the evaluated time points. Figure 3 shows that bias values for all four levels remained consistently within the allowable bias limits of $\pm 15\%$ from T1 to T6. These findings indicate that analytical accuracy was maintained throughout the study duration when T0 was used as the reference. Overall, the accuracy acceptance criterion was fulfilled for all concentration levels in accordance with the manufacturer's performance claims. Low-level calibrators showed minimal bias throughout the study period, remaining largely within ± 50 U/mL of target values.

DISCUSSION

Reagent and quality control (QC) materials demonstrated stable performance for approximately three months, after which increased variability became apparent. Although bias values remained within acceptable limits, the upward trend observed at later time points suggests potential cumulative effects of prolonged storage or repeated instrument exposure. Similar patterns of gradual drift in protein-based immunoassays have been attributed to subtle structural degradation, adsorption effects, or changes in reagent-matrix integrity during extended storage. These findings support cautiously extending storage duration but emphasise the importance of continuous internal quality monitoring when reagents are used beyond manufacturer-recommended periods. Calibrators demonstrated consistent analytical performance across all concentration levels with no evidence of systematic temporal drift. The absence of concentration-dependent instability suggests robust formulation and matrix stability. From a quality assurance perspective, stable calibrator performance strengthens confidence in assay standardisation over time. Importantly, while statistical bias remained acceptable, clinical interpretation must also consider the magnitude of observed variability relative to clinical decision thresholds for KL-6. Although the detected deviations are unlikely to alter clinical classification, laboratories should validate extended storage conditions within their own operational settings before routine implementation. Overall, the findings provide practical laboratory evidence supporting limited extension of storage duration, while reinforcing the need for structured quality oversight.

Several limitations should be acknowledged. First, the study was conducted on a single analytical platform within a single laboratory setting, which may limit generalizability to other instruments or laboratory environments. Second, environmental variables such as repeated onboard temperature fluctuations were not independently controlled beyond routine laboratory practice. Finally, the study design focused on analytical bias and did not evaluate potential long-term clinical impact on patient categorisation. Future multicentre studies incorporating inter-laboratory comparisons and evaluation across different analytical platforms would strengthen the external validity of these findings.

CONCLUSION

Nanopia KL-6 reagents, quality control materials, and calibrators demonstrated acceptable analytical stability during extended storage, with bias remaining within $\pm 15\%$ of baseline values throughout the study period. These findings support the stability of all evaluated materials beyond the manufacturer-recommended period and provide evidence to guide laboratory storage practices and the controlled extension of stability limits without compromising analytical accuracy.

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