Research Article

Validation of the KL6 method on the G600II analyser (Lumipulse) for clinical use in interstitial lung disease

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Article Info

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Keywords

Laboratory method, validation, linearity, precisión, KL-6

Abstract

The Clinical Laboratory (CL) is involved in the prevention, diagnosis and follow-up of disease, as well as in the monitoring of treatment. For this reason, the CL must have robust quality systems in place in order to provide reliable results that help to ensure correct health care. Since the entry into force of the European regulation (IVDR) on in vitro diagnostic medical devices (EU) 2017/746 has generated the loss of CE marking in some laboratory determinations. In our case, Krebs von den Lungen-6 (KL-6), a diagnostic, severity and prognostic marker, as well as a marker of response to treatment, currently has the RUO (research use only) marking and, given its importance in our healthcare environment, we have validated the method with the new reagent in order to be able to continue with the clinical care of patients. In addition, this would keep this analyte within the scope of accreditation. Following the specific CLSI protocols, we carried out a study of precision, linearity as well as the limit of blank and the limit of detection, obtaining results within the limits established by the laboratory. This positive validation of KL6 allows us to continue using this analyte for clinical use and within the scope of accreditation.

Introduction

Lippi and Plebani define Laboratory Medicine as the discipline that deals with the quantitative measurement or qualitative evaluation of any substance in any biological fluid, for diagnostic or research purposes [1]. The results of the measurements obtained are intended to improve the care and/or well-being of the individual and the population. Thus, the Clinical Laboratory (CL) is involved in all aspects of patient care, i.e. from disease prevention, through diagnosis and follow-up, to treatment monitoring. This transversal perspective makes the CL a strategic point in the provision of healthcare, which, together with the growing technological evolution of measurement systems and the involvement of the CL in the diagnostic team, creates the need to review and update the multiple systems used for this purpose [2].

This fundamental task of the CL creates the need to establish robust quality management systems in which the measurement of analytes, applied knowledge and the competencies of the CL staff add value to healthcare by reducing potential laboratory errors and adapting demand management. The ISO 15189:2022 standard applies to all clinical laboratories, including those providing diagnostic, therapeutic and public health services. The aim of this standard is to promote patient

well-being through the quality and competence of clinical laboratories. In order to establish the management of a quality system in the CL, the standard indicates the obligatory nature of the procedures to be applied and the aspects to be taken into account in each of them, but does not specify how to establish them. Responsibility for quality is therefore left to the CL staff, based on knowledge of both the measurement method and the characteristics of the analyte together with the application and clinical repercussions. These quality management systems are dynamic, adapting to the changes that occur in the CL, either internally or externally [3-4]. The process of validating a method or assay involves providing objective evidence indicating compliance with the requirements for the previously defined analytical application. Typically, for in *vitro* diagnostic (IVD) methods, the supplier provides this information and the CL performs a verification of the method to ensure compliance with these requirements within the scope of its population and under its working conditions. Validation of the procedure lies with the CL only when it is a proprietary method or a method exclusively approved for research use only (RUO) [5].

Recently, a new European regulation (IVDR) on in *vitro* diagnostic medical devices, (EU) 2017/746, has come into force, which has meant that some tests which until now had CE marking have not been adapted to this new regulation and can only be used as RUO. This means that if a CL considers its continued use necessary for healthcare purposes, the CL itself will have to carry out this validation process. For this purpose, the Clinical and Laboratory Standards Institute (CLSI) has developed standardized evaluation protocol (EP) reference documents that help CLs to carry out these processes.

Krebs von den Lungen-6 (KL-6), also known as human mucin-1 (MUC-1), is a glycoprotein antigen with a high sialic acid content that is primarily expressed in type II pneumocytes. Due to its high molecular weight, its appearance in the bloodstream results from the destruction of the alveolar epithelium and/or increased capillary permeability [6-8]. Therefore, its blood levels are significantly increased in interstitial lung disease (ILD), a clinical condition characterized by the destruction of lung tissue with inflammation and fibrosis, in contrast to the healthy population and patients with other non-interstitial, non-fibrotic lung diseases or pneumonia [9-10]. Currently, the measurement of KL6, in combination with respiratory function tests and imaging techniques, has been proposed as a diagnostic, severity and prognostic marker [11-12], as well as a marker of treatment response [13-14].

Recently, our supplier of the KL-6 reagent has changed the CE-approved marking to RUO, without any change in the manufacture of the reagent. This change in the marking of the KL-6 reagent triggered the need for our CL to validate the method with the new reagent, in order to continue with the clinical care of patients with interstitial pneumonia, both in the initial diagnosis and in the follow-up of this disease and the potential complications derived from connective tissue diseases. In addition, this would enable the analyte to remain within the scope of accreditation.

Material and methods

The KL-6 validation study was conducted during August 2023 in a tertiary hospital in the Community of Madrid, Spain.

The supplier Fujirebio Europe NV provided Lumipulse® G KL-6 reagent (reference 234594), Lumipulse® PIVKA-II and KL-6 Controls (reference 233900) and Lumipulse® G KL-6 calibrators (Reference 234600).

The validation was performed on the automated platform LUMIPULSE® G600II (Fujirebio) with serial number KF150111B.

The method of determination is a sandwich-type chemiluminescence enzyme immunoassay (CLEIA).

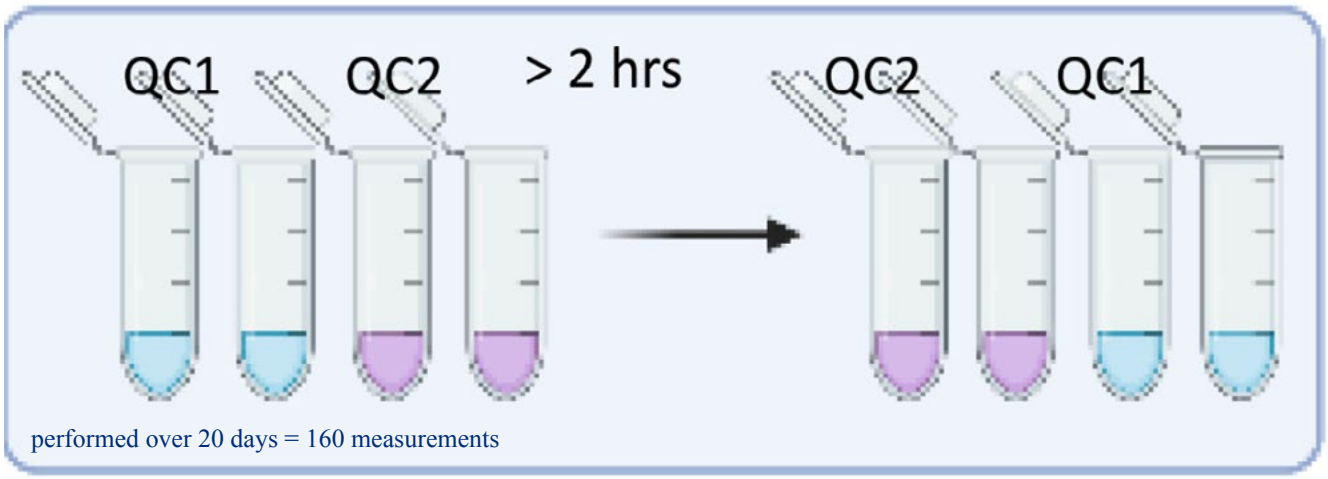
A method validation plan was carried out which included evaluation of precision, linearity, limit of blank and limit of detection of the technique for the RUO-labelled KL-6 reagent, following the relevant Clinical and Laboratory Standards Institute (CLSI) protocols.

Clinical validation in patients was rejected due to previous experience with the analyte, and the CL staff being familiar with its behavior and clinical utility.

1. Evaluation of precision

The CLSI EP05-A3 protocol "Evaluation of Precision of Quantitative Measurement Procedures" [15] was followed. The level 1 control (QC1) and level 2 control (QC2) were analyzed for 20 consecutive days in duplicate with a concentration of 328 and 844 U/mL respectively. This series was repeated twice a day with a time interval of at least 2 hours (Figure 1). The decision limit for total error was set as medical relevance at 10%, as this was the value previously established in our daily quality assurance practice.

Figure 1: Evaluation of precision.



Measurement of the two control levels in duplicate. Repeat this series again after two hours. This is repeated for 20 days for a total of 160 determinations.

2. Evaluation of linearity

The CLSI EP06 protocol "Evaluation of Linearity of Quantitative Measurement Procedures" [16] was followed. Nine concentration levels were evaluated: 44 IU/mL, 346 IU/mL, 698 IU/mL, 951 IU/mL, 1294 IU/mL, 1621 IU/mL, 1954 IU/mL, 2272 IU/mL and 2496 IU/mL. These concentrations were obtained from a pool of patient samples to obtain the lowest and highest concentrations, 44 IU/mL and 2496 IU/mL, respectively. Using these initial concentrations, the above-mentioned protocol was followed by performing the dilutions shown in Table 1 below. The analyte was then analyzed in triplicate for each concentration level in a single test run.

Table 1: Concentrations obtained from the sample pool.

Theoretical concentration (IU/mL)	Sample quantity low concentration (mL)	Sample quantity high concentration (mL)
44 (Low)	1	0
346	0.875	0.125
698	0.750	0.250
951	0.625	0.375
1294	0.500	0.500
1621	0.375	0.625
1954	0.250	0.750
2272	0.125	0.875
2496 (High)	0	1

The different dilutions carried out to obtain the theoretical concentrations

3. Evaluation of limits of blank and detection

The CLSI EP17-A2 protocol "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures" [17] was followed.

The limit of blank (LoB) is defined as the highest apparent concentration of the analyte when replicas of a blank sample without analyte are measured. It refers to the signal/noise of the analyzer and not to the actual concentration of the analyte. For evaluation of the LoB, 60 measurements of the analyte were performed using the zero calibrator as the sample.

The limit of detection (LoD) is the lowest concentration of analyte detectable at a given confidence level, and therefore a sample of known concentration of 26 IU/mL was used for the evaluation of this limit, and measured 60 times (Figure 2).

Figure 2: Evaluation of limit of blank and detection.

3 ST0 tubes x 20 repetitions/1 day = 60 measurements
3 sample tubes 26 IU/mL x 20 repetitions/1 day = 60 measurements

Measurement of three aliquots of calibrator 0 repeated 20 times in one day for a total of 60 determinations. The same series is performed with a sample concentration of 26 IU/mL.

4. Statistical methods

After performing the necessary procedures included in the validation plan, the results obtained were analyzed together with the Quality Department of the Clinical Analysis Service and a report was issued for each result indicating whether the analyte met the previously established acceptance criteria, using the Analyse-it v6.15 program.

The decision limit for total error was set as medical relevance at 10%, as this was the value previously established in our daily quality assurance practice.

Results

Evaluation of precision. The results obtained from the analysis are within the precision limits established by the laboratory of 10% at the two concentration levels studied, 328 IU/mL and 844 IU/mL, with a coefficient of variation of 3.4% and 2.7% respectively.

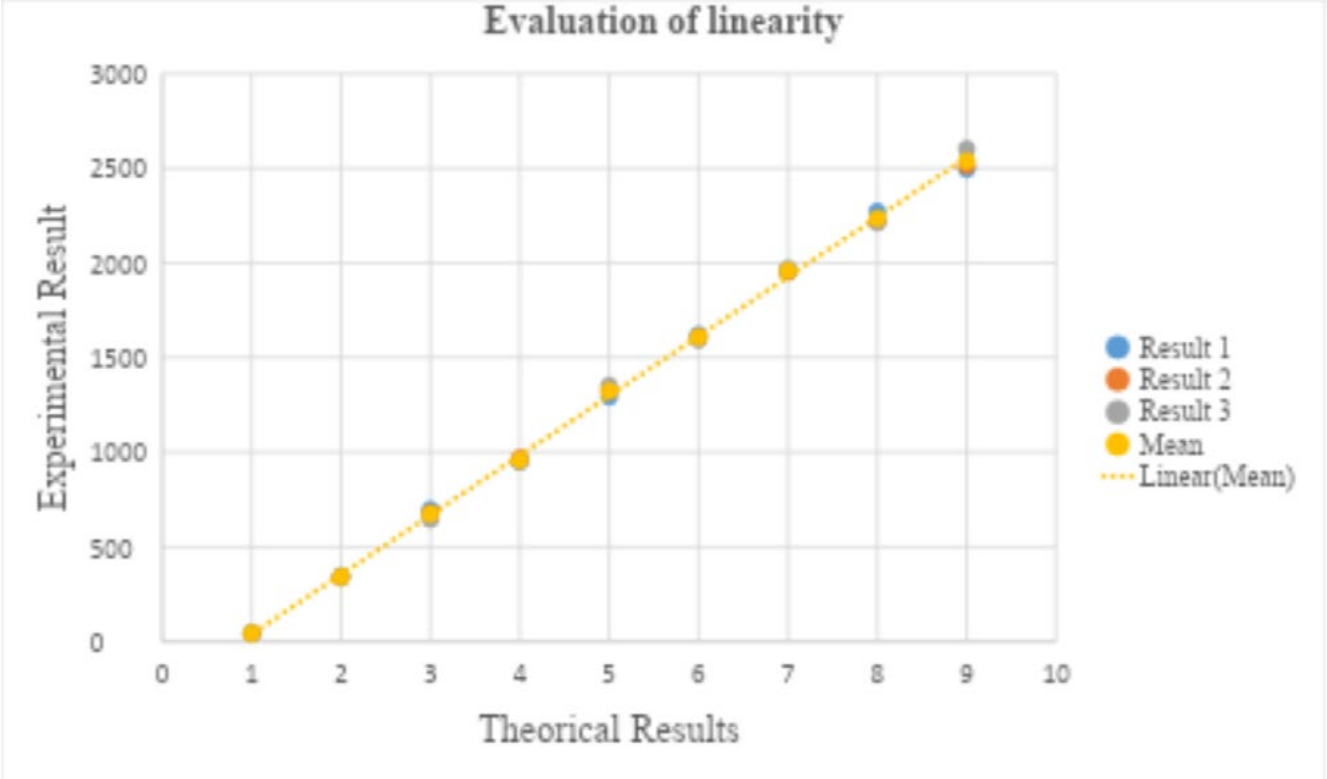
Table 2: Results of evaluation of precision.

Precision				
Theoretical concentration (IU/mL)	**Average concentration (IU/mL)**	**Coefficient of variation (CV)**	**Standard deviation (SD)**	**Allowable SD/CV**
328	355.3	3.4 %	12.2	10%
844	913.2	2.7 %	24.6	10%

The CVs and SDs obtained at the two concentrations at which the accuracy was studied.

Evaluation of linearity range. The results obtained from the analysis, setting the precision limit at 10%, indicate that the method studied is linear from a concentration of 44 IU/mL to 2496 IU/mL (Figure 3). Samples with a concentration below 44 IU/mL shall be reported as <44 IU/mL and samples with a concentration above 2496 IU/mL shall be diluted (Table 3).

Figure 3: Evaluation of linearity.



Graph generated from the results obtained after the evaluation of linearity.

Table 3: Results of the evaluation of linearity.

Theoretical result	Mean	Linear fit	Nonlinearity	90% familywise CI (98,89% individual CI)	Allowable nonlinearity
44.000	43.0	39.1	10.0*	-87.6% to 107.6%	±10.0%
359.625	341.7	353.7	-3.4%	-14.2% to 7.4%	±10.0%
675.250	674.0	668.4	0.8%	-4.9% to 6.5%	±10.0%
990.875	957.3	983.0	-2.6%	-6.5% to 1.3%	±10.0%
1306.500	1324.0	1297.7	2.0%	-0.9% to 5.0%	±10.0%
1622.125	1607.3	1612.3	-0.3%	-2.7% to 2.1%	±10.0%
1937.750	1959.0	1927.0	1.7%	-0.3% to 3.6%	±10.0%
2253.375	2235.0	2241.6	-0.3%	-2.0% to 1.4%	±10.0%
2569.000	2537.7	2556.2	-0.7%	-2.2% to 0.8%	±10.0%

*Performance requirement not met
Results obtained in the evaluation of linearity at each concentration level studied

Evaluation of limit of blank and detection. The results obtained from the analysis showed that the limit of quantification is 26 IU/mL with imprecision of 3.7%, enabling differentiation of the concentration of the samples from the zero concentration with a coefficient of variation of 5.2% and reporting of patient results as <26 IU/mL, as shown in Table 4.

Table 4: Results of the absorbances.

Precision			
Theoretical concentration (IU/mL)	**Mean absorbance**	**Coefficient of variation (CV)**	**Standard deviation (SD)**
0	720.8	5.2 %	37.3
26	10400.3	3.7 %	381.7

Results of the absorbances obtained at theoretical concentrations of 0 and 26 IU/mL.

Discussion

Occasionally, changes in the internal policies of the suppliers of the reagents used in the CL lead to changes in the activity of the laboratory staff. In our case, removal of the CE marking from the KL-6 reagent and its switch to RUO required the CL staff to evaluate the requirements and specifications necessary to validate the KL-6 method and thus continue with the clinical care of patients treated in our healthcare area.

After evaluation of the results obtained from the precision study, it can be said that the KL-6 measurement does not exceed the limit of precision of 10% established by the laboratory for the two concentration levels studied, 328 and 844 (IU/mL), and therefore complies with the CL's quality assurance. Furthermore, based on the results of the limit of blank and detection evaluation, it can be established that the reagent used in the LUMIPULSE platform (Fujirebio) is able to differentiate the background noise of the analyzer from the concentration of the analyte and to measure a KL-6 concentration of 26 IU/mL with a coefficient of variation of less than 10%, specifically 3.7%.

Regarding the linearity range of the technique, we studied the concentration range from 44 IU/mL to 2496 IU/mL, and were able to establish that it is linear in this range. Thus, concentrations below 44 IU/mL should be reported as <44 IU/mL and concentrations above 2496 IU/mL should be diluted. The choice of this range arose for three fundamental reasons, firstly because of the availability in the CL of the pool of serum samples from patients with these concentrations, secondly because the dilution recommendations of the CLSI protocol for the linearity range should be followed, and finally because the clinical decision level of KL-6 for healthy versus pathological discrimination was known and established as 500 IU/mL according to the literature. It is worth noting that although we know that at KL-6 concentrations of 26 IU/mL the inaccuracy is less than 10%, we report KL-6 results as less than 44 IU/mL because we have studied linearity in the range 44-2496 IU/mL and we do not know if it meets linearity criteria between the concentrations of 26-44 IU/mL. In addition, the disease associated with this analyte is produced by elevation of its concentration, with no clinical repercussions at the previously mentioned concentration levels of 26 or 44 IU/mL.

Another point that we consider important in our laboratory is the loss of ISO 15189:2022 accreditation of KL-6, due to the RUO marking of this reagent. After this validation, which covers the different analytical quality aspects required for the accreditation of this test, in the next external audit we will declare the evaluation of this analyte in order to obtain its accreditation.

Conclusions

In conclusion, it can be stated that the RUO-labelled KL-6 reagent measured on the LUMIPULSE platform (Fujirebio) meets the quality assurance criteria established in our laboratory and can be used in routine clinical practice, although it does not have the IVDR marking.

Having carried out an experimental design following CLSI protocols means that the results obtained could be useful for other clinical laboratories interested in incorporating this analytical method into their healthcare service portfolio.

Limitations

An assessment of the range of linearity between the KL-6 concentration of 26 IU/mL and 44 IU/mL would be necessary in order to report patient results as below 26 IU/mL, although at these levels there is no clinical impact and therefore patient management does not change.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

Ethical Considerations

This study did not involve patients and therefore no declaration of ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki, is required.

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