

Assessment of the degree of adherence of medical laboratories to KDIGO 2012 guideline for evaluation and management of CKD in Czechia and Slovakia

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Abstract

Introduction: The aim of the study is to assess the degree of adherence of medical laboratories to Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (CKD) in laboratory practice in Czechia and Slovakia.

Materials and methods: An electronic questionnaire on adherence to KDIGO 2012 guideline was designed by an external quality assessment (EQA) provider SEKK spol. s.r.o. The questionnaire was placed and distributed through website to all medical biochemistry laboratories in Czechia and Slovakia (N = 396).

Results: A total of 212 out of 396 laboratories responded to the questions, though some laboratories only answered some questions, those applicable to their practice. A total of 48 out of 212 laboratories adopted the KDIGO 2012 guideline in full extent. The metrological traceability of creatinine measurement to standard reference material of SRM 967 was declared by 180 out of 210 laboratories (two of the responding laboratories did not measure creatinine). Thirty laboratories are not well educated on traceability of creatinine measurement and seven laboratories do not calculate estimated glomerular filtration rate (eGFR). Both urinary albumin concentration and albumin to creatinine ratio are reported by 144 out of 175 laboratories (37 of the responding laboratories did not measure urinary albumin).

Conclusion: Majority of laboratories in Czechia and Slovakia adopted some parts of the KDIGO 2012 guideline in their practice, but only 23% of the laboratories apply them completely. Thus, further education and action should be conducted to improve its implementation.

Keywords: creatinine; cystatin C; glomerular filtration; estimated glomerular filtration rate; post-analytical phase

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Introduction

The current definition of chronic kidney disease (CKD) includes an estimated glomerular filtration rate (eGFR) decreased below 60 mL/min/1.73m² and/or the presence of kidney damage for a time period longer than 3 months with implications for health (1). The overall prevalence of CKD was 11.6% in China and 12.9% in the United States (2). The accurate prevalence of CKD in Czechia and Slovakia is not known. In Europe, the adjusted CKD stages

1–5 prevalence varies between 3.31% in Norway and 17.3% in northeast Germany (3). The risk factors for development of CKD are defined. They are: age, hypertension, diabetes, obesity, cardiovascular disease and others. Simple obesity increases the risk of CKD independently of dyslipidaemia, diabetes, and hypertension (4). Correct diagnosis of CKD strongly depends on serum creatinine, cystatin C and urinary albumin measurement. Albu-

minuria usually precedes the decline of kidney function (5). Clinical laboratories are recommended to measure creatinine by enzymatic method traceable to standard reference material SRM 967 (6). Cystatin C should be measured with traceability of measurement to international reference material DA ERM 471 (7). Patients with CKD are at high or very high cardiovascular risk and have lower low density lipoprotein (LDL) cholesterol treatment targets (8). Identification of these high risk patients is the main reason why Kidney Disease Improving Global Outcomes (KDIGO) 2012 guideline should be implemented. The Czech Society for Clinical Biochemistry and Czech Nephrology Society created Guidelines on diagnostics of Chronic Kidney Disease in 2014. Key points of KDIGO 2012 guidelines are included but the unit of eGFR is changed from mL/min to mL/s (9). The intention of this guideline was to harmonize creatinine and cystatin C measuring and eGFR reporting. The guideline strongly recommends using standardized creatinine and cystatin C methods in laboratories. Many articles in this field were published in Czech national journal of Clinical Biochemistry and Metabolism. This topic was also repeatedly discussed by external quality assessment (EQA) supervisors in EQA reports. Before the publication of these guidelines there was lower level of metrological traceability of creatinine measurement and the modification of diet in renal disease (MDRD) equation was the most common equation for estimated glomerular filtration rate from serum creatinine (eGFR_{crea}). Patient safety, improvement of the level of standardization of measurement and improvement of the harmonization of reporting of laboratory test results are reasons why we performed this survey. The aim of the study is to assess the degree of adherence of medical laboratories to KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of CKD in laboratory practice in Czechia and Slovakia.

Materials and methods

Study design

An electronic questionnaire on adherence to KDIGO 2012 guideline was designed by an EQA provider SEKK spol. s.r.o., with registered office in Pardubice, Czechia. The questionnaire was placed and distributed through EQA SEKK's website www.sekk.cz and sent by e-mail to all medical biochemistry laboratories in Czechia and Slovakia (N = 396) as a part of the basic clinical chemistry EQA scheme. The questions of the questionnaire on adherence to KDIGO 2012 guideline in Czechia and Slovakia are shown in Table 1. Information on the survey and instructions for completing the questionnaire were part of the EQA scheme. Laboratory directors had responsibility for data entry and data were collected in the period from January 7th to January 25th 2019. The entry of results could be monitored by a link to the website. Participation in the survey was voluntary. In addition to the questionnaire, data on the type of creatinine method used among laboratories were collected from the results of first basic clinical chemistry EQA scheme (the number of participants was 188). Information on the type of creatinine method was not derived from the survey. These data were collected as a creatinine enzymatic method is recommended by KDIGO 2012 guideline. In the end, 212 laboratories participated in the study. All data were collected and no laboratory response was excluded. We did not compare results among different kinds of laboratories. The ideal target is full guideline implementation. Full guideline implementation was defined as the adoption of the KDIGO 2012 guideline in full extent including cystatin C traceable method and eGFR. Partial guideline implementation was defined as creatinine measurement traceable to standard reference material SRM 967. After the survey had been completed, all participants received interpretative comments from the EQA supervisors, containing references to related pages of the KDIGO 2012 guideline. These comments were not part of the questionnaire.

TABLE 1. The list of questions of the questionnaire on adherence to KDIGO 2012 guideline among laboratories in Czechia and Slovakia

Question	Possible answers
1. Do you measure serum creatinine concentrations?	<ul style="list-style-type: none"> • No • Yes
2. Do you perform serum cystatin C measurement?	<ul style="list-style-type: none"> • No • Yes
3. Do you measure albumin in urine?	<ul style="list-style-type: none"> • No • Yes
4. Do you calculate eGFR? (3 options possible)	<ul style="list-style-type: none"> • No • From serum creatinine • From serum cystatin C • Combined estimation from serum creatinine and cystatin C
5. What information on metrological traceability does the manufacturer of your creatinine method provide?	<ul style="list-style-type: none"> • Traceability to SRM 967 • Traceability is not declared • Information is not available
6. Which equation do you use for eGFR_{crea}?	<ul style="list-style-type: none"> • CKD-EPI from 2009 • MDRD equation • Another equation (provide)
7. In which subgroups of patients do you report eGFR_{crea}?	<ul style="list-style-type: none"> • In all patients • On request
8. In which subgroups of patients do you not report eGFR_{crea}?	<ul style="list-style-type: none"> • Acute care • Oncology patients • Pregnant women • Another subgroup (provide information)
9. What decision limit of eGFR_{crea} do you use?	<ul style="list-style-type: none"> • 60 mL/min • 90 mL/min • Another (provide information)
10. What information on metrological traceability does the manufacturer of your cystatin C method provide?	<ul style="list-style-type: none"> • Traceability to international reference material DA ERM 471 • Traceability is not declared • Information is not available
11. Which equation do you use for eGFR_{cys}?	<ul style="list-style-type: none"> • CKD-EPI from 2012 • CAPA equation • Another equation (provide)
12. In which subgroups of patients do you report eGFR_{cys}?	<ul style="list-style-type: none"> • In all patients • On request
13. In which subgroups of patients do you not report eGFR_{cys}?	<ul style="list-style-type: none"> • provide information
14. How do you calculate eGFR?	<ul style="list-style-type: none"> • By laboratory information system • By web calculator (specify) • By excel • We use different way (provide information)
15. Which information do you report when you determine albuminuria?	<ul style="list-style-type: none"> • Only urinary albumin concentration • Only albumin to creatinine ratio • Both urinary albumin concentration and albumin to creatinine ratio • We use different procedure (describe)
16. Which way for albuminuria determination prevails in your laboratory?	<ul style="list-style-type: none"> • Spot urine samples • Urine timed collection

TABLE 1. (continued)

17. How many decimal places do you use for creatinine concentration reporting in $\mu\text{mol/L}$?	<ul style="list-style-type: none"> • We do not control it, it depends on laboratory information system • Whole numbers • 1 decimal place • 2 decimal places • It depends on uncertainty of measurement
18. How many decimal places do you use for cystatin C concentration reporting in mg/L?	<ul style="list-style-type: none"> • We do not control it, it depends on laboratory information system • 1 decimal place • 2 decimal places • It depends on uncertainty of measurement

KIDGO – Kidney Disease Improving Global Outcomes. eGFR - estimated glomerular filtration rate. SRM - standard reference material. CKD-EPI - chronic kidney disease epidemiology collaboration. eGFR_{crea} - glomerular filtration rate estimated from serum creatinine. MDRD - modification of diet in renal disease. eGFR_{cys} - glomerular filtration rate estimated from serum cystatin C. CAPA - Caucasian, Asian, Paediatric, Adult.

Statistical analysis

Data were collected to Microsoft Excel Office 2007 program (Microsoft, Washington, USA). The total absolute number of specific responses of each specific question and their relative percentages compared to the total number of responses to the particular question were calculated. The denominator of the percentages for full and partial guideline implementation was the total number of participants of the survey (N = 212).

Results

The response rate to the questionnaire was 54% (212 out of 396 laboratories). The answers to the questionnaire by laboratory participants on adherence to KDIGO 2012 guideline are provided in Table 2. Of all participants, 210 laboratories measured serum creatinine, 57 serum cystatin C, and 175 measured urinary albumin. A total of 53% (99/188) laboratories measured creatinine by enzymatic method. The Jaffe method was measured by

TABLE 2. Answers to the questionnaire by laboratory participants on adherence to KDIGO 2012 guideline

Question	Total answers (N)	Answer – number of laboratories, N (%)
1. Do you measure serum creatinine concentrations?	212	<ul style="list-style-type: none"> • No – 2 (1) • Yes – 210 (99)
2. Do you perform serum cystatin C measurement?	208	<ul style="list-style-type: none"> • No – 151 (73) • Yes – 57 (27)
3. Do you measure albumin in urine?	210	<ul style="list-style-type: none"> • No – 35 (17) • Yes – 175 (83)
4. Do you calculate eGFR? (3 options possible)	199	<ul style="list-style-type: none"> • No – 7 (4) • From serum creatinine – 192 (96) • From serum cystatin C – 41 (21) • Combined estimation from serum creatinine and cystatin C – 24 (12)
5. What information on metrological traceability does the manufacturer of your creatinine method provide?	195	<ul style="list-style-type: none"> • Traceability to SRM 967 – 180 (92) • Traceability is not declared – 8 (4) • Information is not available – 7 (4)

TABLE 2. (continued)

6. Which equation do you use for eGFR_{crea}?	197	<ul style="list-style-type: none"> • CKD-EPI from 2009 – 148 (75) • MDRD equation – 42 (21) • Another equation – 7 (4) (6x Lund-Malmö, combination of equations above)
7. In which subgroups of patients do you report eGFR_{crea}?	197	<ul style="list-style-type: none"> • In all patients – 139 (71) • On request – 58 (29)
8. In which subgroups of patients do you not report eGFR_{crea}?	84	<ul style="list-style-type: none"> • Acute care – 7 (8) • Oncology patients – 2 (3) • Pregnant women – 21 (25) • Another subgroup – 54 (64) (patients on dialysis, with creatinine above 250 $\mu\text{mol/L}$)
9. What decision limit of eGFR_{crea} do you use?	176	<ul style="list-style-type: none"> • 60 mL/min – 64 (36) • 90 mL/min – 100 (57) • Another 12 (7) (9x 66 mL/min, 2x 120 mL/min, 1x 69 mL/min)
10. What information on metrological traceability does the manufacturer of your cystatin C method provide?	57	<ul style="list-style-type: none"> • Traceability to international reference material ERM DA 471 – 51 (89) • Traceability is declared only generally – 4 (7) <ul style="list-style-type: none"> • Information is not available – 2 (4)
11. Which equation do you use for eGFR_{cys}?	50	<ul style="list-style-type: none"> • CKD-EPI from 2012 – 48 (96) <ul style="list-style-type: none"> • CAPA – 1 (2) • Another equation – 1 (2) (equation by Grubb)
12. In which subgroups of patients do you report eGFR_{cys}?	51	<ul style="list-style-type: none"> • In all patients – 42 (82) • On request – 9 (18)
13. In which subgroups of patients do you not report eGFR_{cys}?	3	<ul style="list-style-type: none"> • Acute care – 0 (0) • Oncology patients – 1 (33) • Pregnant women – 2 (67)
14. How do you calculate eGFR?	186	<ul style="list-style-type: none"> • By laboratory information system – 184 (99) <ul style="list-style-type: none"> • By web calculator – 0 (0) • By excel – 2 (1)
15. Which information do you report when you determine albuminuria?	175	<ul style="list-style-type: none"> • Only urinary albumin concentration – 16 (9) • Only albumin to creatinine ratio – 15 (9) • Both urinary albumin concentration and albumin to creatinine ratio – 144 (82)
16. Which way for albuminuria determination prevails in your laboratory?	169	<ul style="list-style-type: none"> • Spot urine samples – 155 (92) • Urine timed collection – 14 (8)
17. How many decimal places do you use for creatinine concentration reporting in $\mu\text{mol/L}$?	198	<ul style="list-style-type: none"> • We do not control it, it depends on laboratory information system – 13 (7) <ul style="list-style-type: none"> • Whole numbers – 116 (59) • 1 decimal place – 56 (28) • 2 decimal places – 13 (7) • It depends on uncertainty of measurement – 0 (0)
18. How many decimal places do you use for cystatin C concentration reporting in mg/L?	59	<ul style="list-style-type: none"> • We do not control it, it depends on laboratory information system – 2 (3) <ul style="list-style-type: none"> • 1 decimal place – 6 (10) • 2 decimal places – 51 (86) • It depends on uncertainty of measurement – 0 (0)

KIDGO – Kidney Disease Improving Global Outcomes. eGFR - estimated glomerular filtration rate. SRM - standard reference material. CKD-EPI - chronic kidney disease epidemiology collaboration. eGFR_{crea} - glomerular filtration rate estimated from serum creatinine. MDRD - modification of diet in renal disease. eGFR_{cys} - glomerular filtration rate estimated from serum cystatin C. CAPA - Caucasian, Asian, Paediatric, Adult.

89 laboratories. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used by 75% of all laboratories which were calculating eGFR_{crea}. Both urinary albumin concentration and albumin to creatinine ratio are reported by 144 out of 175 laboratories (37 of the responding laboratories did not measure urinary albumin).

A total of 48 out of 212 laboratories adopted the KDIGO 2012 guideline in full extent including cystatin C traceable method and eGFR. The partial KDIGO 2012 guideline implementation was found in 180 out of 212 (85%) laboratories. The interpretative comments provided by the EQA supervisors after the survey are shown in Table 3.

TABLE 3. Interpretative comments by supervisors on questions related to KDIGO 2012 guideline adherence among laboratories in Czechia and Slovakia after the survey

Question text	Educational comment – recommendation by KDIGO 2012 Clinical Practice Guideline and according to current knowledge
1. Do you measure serum creatinine concentrations?	–
2. Do you perform serum cystatin C measurement?	–
3. Do you measure albumin in urine?	–
4. Do you calculate eGFR? (3 options possible)	KDIGO guideline recommends reporting eGFR together with concentration of creatinine and or cystatin C (page 7 of the KDIGO guideline).
5. What information on metrological traceability does the manufacturer of your creatinine method provide?	KDIGO guideline recommends method traceable to international standard reference material for measurement of serum creatinine (page 39 of the KDIGO guideline).
6. Which equation do you use for eGFR _{crea} ?	KDIGO guideline recommends CKD-EPI equation from 2009 (page 7 of the KDIGO guideline), MDRD equation is validated only for patients with CKD.
7. In which subgroups of patients do you report eGFR _{crea} ?	eGFR _{crea} was developed for hemodynamically stable non–acute patients without muscle loss. KDIGO guideline recommends eGFR _{crea} for initial assessment of GFR in most circumstances (page 38 of the KDIGO guideline).
8. In which subgroups of patients do you not report eGFR _{crea} ?	No eGFR _{crea} equation was validated for pregnant women and acute care patients. But physicians still need to dose a drugs excreted by kidneys. KDIGO guideline recommends eGFR _{cys} for confirmatory testing in specific circumstances when eGFR based on serum creatinine is less accurate (page 38 of the KDIGO guideline).
9. What decision limit of eGFR _{crea} do you use?	The limit of 60 mL/min defines CKD by GFR criterion The limit of 90 mL/min defines normal GFR (page 5 of the KDIGO guideline)
10. What information on metrological traceability does the manufacturer of your cystatin C method provide?	KDIGO guideline recommends standardized method traceable to DA471/IFCC for measurement of serum cystatin C (page 51 of the KDIGO guideline).
11. Which equation do you use for eGFR _{cys} ?	KDIGO guideline recommends CKD-EPI equation from 2012 (page 51 of the KDIGO guideline). The CAPA equation was also validated.
12. In which subgroups of patients do you report eGFR _{cys} ?	eGFR _{cys} was developed for hemodynamically stable non–acute patients (page 50 of the KDIGO guideline).
13. In which subgroups of patients do you not report eGFR _{cys} ?	No eGFR _{cys} equation was validated for pregnant women and acute care patients (page 50 of the KDIGO guideline.) But physicians still need to dose a drugs excreted by kidneys.
14. How do you calculate eGFR?	The calculation by laboratory information system is probably the most practical approach.
15. Which information do you report when you determine albuminuria?	The reporting of albumin to creatinine ratio in addition to albumin concentration is recommended by KDIGO guideline (page 56 of the KDIGO guideline).

TABLE 3. (continued)

16. Which way for albuminuria determination prevails in your laboratory?	The spot urine sample is probably an easier and more practical approach compared to urine timed collection with the risk of incomplete collections. Untimed urine samples are included in the KDIGO guideline (page 56 of the KDIGO guideline).
17. How many decimal places do you use for creatinine concentration reporting in $\mu\text{mol/L}$?	KDIGO guideline recommends that serum creatinine concentration should be rounded to the nearest whole number in $\mu\text{mol/L}$ (page 39 of the KDIGO guideline).
18. How many decimal places do you use for cystatin C concentration reporting in mg/L?	KDIGO guideline recommends reporting cystatin C results rounded to two decimal places in mg/L (page 51 of the KDIGO guideline).

KIDGO – Kidney Disease Improving Global Outcomes. eGFR – estimated glomerular filtration rate. SRM – standard reference material. CKD-EPI – chronic kidney disease epidemiology collaboration. eGFR_{crea} – glomerular filtration rate estimated from serum creatinine. MDRD – modification of diet in renal disease. eGFR_{cys} – glomerular filtration rate estimated from serum cystatin C. CAPA – Caucasian, Asian, Paediatric, Adult.

Discussion

We have analysed the degree of adherence of medical laboratories to KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of CKD in laboratory practice. A total of 48 out of 212 laboratories adopted the KDIGO 2012 guideline in full extent including cystatin C traceable method and eGFR. Partial KDIGO 2012 guideline implementation was found in 180 out of 212 (85%) laboratories. Biljak *et al.* performed a similar study with the purpose to improve education of laboratories and harmonization of KDIGO 2012 guideline implementation in Croatia (10). In our study a larger proportion of laboratories reported eGFR, the MDRD equation was less frequent, and 24 hour urine timed collection was also less frequent compared to the Croatian study. The higher proportion of MDRD equation in the study by Biljak *et al.* may be explained by the fact that their work was performed sooner after KDIGO guidelines reporting and MDRD equation preceded the CKD-EPI one. Drion *et al.* analysed national data from the Dutch external quality organization and concluded that enzymatic determination has less variability in serum creatinine measurement than Jaffe techniques, and therefore results in more accurate staging of CKD (11). There were 47% of laboratories with creatinine method based on non-specific Jaffe reaction in our study. We hope that this number will be decreasing in the future due to further education. The Reference Institute of Bioanalytics, German proficiency testing organization, showed

in the last external quality assessment survey that 453 out of 635 (71%) laboratories used Jaffe method for creatinine determination (12). There was lower proportion of creatinine determination by Jaffe method in our study. Jaffe methods for the creatinine determination should be replaced by enzymatic measurement in medical laboratories.

Many patients have lost their muscle mass due to chronic conditions such as oncology disease. The study by Šálek *et al.* reported overestimation of glomerular filtration rate (GFR) by eGFR_{crea} compared to isotopic reference method in oncology patients before cisplatin treatment (13). Two laboratories did not calculate eGFR_{crea} in oncology patients in our study.

The KDIGO 2012 guideline recommends confirmation of CKD by estimated glomerular filtration rate from serum cystatin C (eGFR_{cys}) when eGFR_{crea} is below $60 \text{ mL/min/1.73m}^2$. Only 27% of laboratories in our survey measure cystatin C which enables estimation of GFR in patients with muscle loss. We plan further data analysis and investigations regarding eGFR_{cys}. KDIGO 2012 guideline recommends CKD-EPI equation for estimation of eGFR_{cys} with standardized cystatin C measurement. The Caucasian, Asian, Paediatric, Adult (CAPA) equation is the new alternative for estimation of GFR from standardized serum cystatin C concentration (14). Only a single participant used the CAPA equation in our survey. Current clinical

practice relies on eGFR_{crea} but eGFR_{cys} may be useful for patients who lost their muscles due to chronic diseases.

The MDRD study group which enrolled 1628 patients with CKD was used for the development of the MDRD eGFR_{crea} equation (15). The limitations of the MDRD study were that creatinine was measured by nonspecific Jaffé reaction and participants from general population were not included. Forty two laboratories in our study still used the MDRD equation. Fuček *et al.* showed that eGFR based on CKD-EPI equations correlated significantly with endemic nephropathy (16). Another new option for eGFR_{crea} is the Lund-Malmö equation (17). In our survey the CKD-EPI equation was used by 75% of all laboratories which were calculating eGFR_{crea}.

The work by Manns *et al.* demonstrated that low number of patients with CKD had albuminuria measurement. They found the care gap among all patients with CKD. Authors suggest the measurement of albumin to creatinine ratio in patients at risk for CKD development as a quality indicator (18). The urinary albumin to creatinine ratio is more practical than urine timed collection. A majority of laboratories in our study (92%) calculated mainly albumin to creatinine ratio from untimed urine sample compared to urine timed collection. Fung *et al.* reported that combining eGFR and albumin to creatinine ratio level was more accurate in predicting risk of cardiovascular disease and all-cause mortality. Serum creatinine with calculation of eGFR_{crea} and urinary albumin to creatinine ratio should be regularly monitored in diabetic patients. Early intervention to halt or even reverse the progression reduces the risk of cardiovascular disease and all-cause mortality. The findings call for more aggressive screening and intervention of albuminuria in diabetic patients (19). It means that all laboratories which measure urine albumin should also calculate albumin to creatinine ratio. In this survey a total of 91% (159 out of 175) of laboratories that measure urine albumin calculate also albumin to creatinine ratio. The ideal state would be that all biochemistry laboratories measure both

creatinine as the marker of kidney function and urinary albumin as the marker of kidney damage. The reason is that the marker of kidney damage precedes the decline of kidney function.

The number of decimal places in reporting serum creatinine and cystatin C concentrations is the issue of uncertainty of measurement. Each series of calibrator should be accompanied with the information on its uncertainty (20). Kidney Disease Improving Global Outcomes 2012 guideline recommends that serum creatinine concentration should be reported and rounded to the nearest whole number when the unit of measurement is $\mu\text{mol/L}$. It is recommended to report and round serum cystatin C concentration in mg/L to two decimal places when the unit of measurement is mg/L (21). In this survey 59% laboratories reported serum creatinine as whole numbers and 86% reported serum cystatin C rounded to two decimal places. Biljak *et al.* reported 6 key factors for laboratories implementing the national guidelines for the diagnosis and management of CKD. The first factor is good communication between laboratory and clinicians (22). We also share the opinion that open and frequent communication is important for any guideline implementation.

The limitations of the study are low survey response rate and not asking about participants' creatinine method in the survey, but obtaining it in a different way instead. Further, some participants did not respond to all questions. Information from participants may not reflect the real situation. We were not able to compare results of hospital laboratories, specialized centre laboratories, and private laboratories. In summary, a majority of laboratories in Czechia and Slovakia adopted some parts of the KDIGO 2012 guideline in laboratory practice but there is still further need of education on traceability of measurement, the importance of eGFR calculation, and harmonization of reporting of results in some cases.

Potential conflict of interest

None declared.

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