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Technical Report

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# A short guideline on chronic kidney disease for medical laboratory practice

## Kronik böbrek hastalığında tıbbi laboratuvar uygulamaları için kısa kılavuz

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**Abstract:** Chronic kidney disease (CKD) is asymptomatic in the early stage. Kidney function might be lost 90% when the symptoms are overt. However, in case of early detection, progression of the disease can be prevented or delayed. If not detected it results in end stage renal disease. Therefore, the level of awareness about CKD should be increased. The role of medical laboratory is utmost important for the diagnosis and staging of CKD. In this paper, the main tasks of the laboratory specialists are described and the outlines are as follows.

• Creatinine assays should be traceable to internationally recognised reference materials and methods, specifically isotope dilution mass spectrometry.

• When reporting the creatinine result, eGFR should also be reported in adult (>18 years) population. A warning expression should be included in the report form if eGFR result is <60 mL/min/1.73 $m^2$.

• eGFR values should be expressed quantitatively up to 90 mL/min/1.73 $m^2$ by CKD-EPI equation. Above 90 mL/min/1.73 $m^2$, eGFR values can be expressed quantitatively or >90 mL/min/1.73 $m^2$.

• eGFR equations of the adult population should not be used for pediatric population. Different equations utilizing also patient height should be used. The enzymatic creatinine assay should be preferred. eGFR based on cystatin C can be used for confirmation in the pediatric population.

• Cystatin C measurements, at least when eGFR based on creatinine is not reliable and for confirmation should be encouraged.

• Proteinuria or albuminuria values should be measured in spot samples and reported in proportion to creatinine.

**Keywords:** Albumin, Chronic kidney disease, Creatinine, Cystatin C, Glomerular filtration rate, Urine

**Özet:** Kronik böbrek hastalığı (KBH) erken dönemde asemptomatiktir. Semptomlar ortaya çıktığında böbrek fonksiyonu %90 oranında azalmış olabilir. Buna karşı-

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lık, eğer erken dönemde saptanırsa hastalığın ilerlemesi önlenebilir veya geciktirilebilir. Eğer saptanamazsa, KBH son dönem böbrek yetmezliği ile sonuçlanır. Bu yüzden, KBH hakkında farkındalık artırılmalıdır. KBH tanısı ve derecelendirmesinde tıbbi laboratuvarın rolü son derece önemlidir. Bu derlemede, laboratuvar uzmanlarının temel görevleri tanımlandı. Bunlar anahatlarıyla aşağıda verilmektedir:

• Kreatinin ölçümleri uluslararası geçerliliği olan referans materyallere ve izotop seyreltimi kütle spektrometri gibi referans yöntemlere göre izlenebilir olmalıdır.

• Kreatinin sonuçları rapor edilirken, erişkin popülasyonda (>18 yaş) eGFR de verilmelidir. Eğer eGFR sonucu <60 mL/min/1.73 $m^2$ ise raporda bir uyarı ifadesi yer almalıdır.

• eGFR sonuçları CKD-EPI eşitliği ile 90 mL/min/1.73 $m^2$'ye kadar kantitatif olarak verilmelidir; 90 mL/min/1.73 $m^2$'nin üzerindeki değerler kantitatif olarak veya >90 mL/min/1.73 $m^2$ olarak ifade edilebilir.

• Erişkin popülasyon eGFR değerleri pediyatrik popülasyon için kullanılmamalıdır. Pediyatrik popülasyon için hasta boyunu da içeren farklı eşitlikler kullanılmalıdır ve enzimatik kreatinin yöntemi tercih edilmelidir. Sistatin C'ye dayanan eGFR değeri pediyatrik popülasyonda doğrulama amaçlı kullanılabilir.

• eGFR'ye dayalı kreatinin sonucu güvenilir olmadığında ve doğrulama gerektiğinde sistatin C ölçümleri teşvik edilmelidir.

• Proteinüri ve albüminüri değerleri rastgele örneklerde ölçülmeli ve kreatinine oranlanarak verilmelidir.

**Anahtar Kelimeler:** Albümin, Kronik böbrek hastalığı, Kreatinin, Sistatin C, Glomerüler filtrasyon hızı, İdrar

# 1 Introduction

Chronic kidney disease (CKD) is an important public health problem in Turkey like in all over the World. CKD is asymptomatic in the early stage. Kidney function might be lost 90% when the symptoms are overt. However, in case of early detection, progression of the disease can be prevented or delayed. If not detected it results in end stage renal disease (ESRD). Statistics show that the number of patients with ESRD in our country will exceed 100 000 in 2016. Therefore, the level of awareness should be increased. Awareness level is <10% in the World [1] and not more than 2% in Turkey [2].

The role of medical laboratory is utmost important for the diagnosis and staging of CKD. According to Kidney Disease Improving Global Outcomes, Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (KDIGO 2012), CKD is defined as abnormalities of kidney structure or function, present for >3 months. CKD diagnosis cannot be confirmed if duration is <3 months; but abnormality results from CKD or acute kidney injury [3]. These abnormalities are decreased glomerular filtration rate (<60 mL/min/1.73 $m^2$) for any reason, albuminuria, urine sediment abnormalities (for example hematuria due to causes other than urologic), electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, and history of kidney transplantation [3,4]. GFR and urine albumin categories are used for CKD staging in the guideline (Table 1).

The importance of some laboratory tests is seen in screening for CKD as well as diagnosis, evaluation, staging and monitoring. GFR can be calculated (esti-

**Table 1:** Staging of CKD according to GFR and albuminuria categories [3,4].

| GFR category | GFR (mL/min/1.73 $m^2$) | | Terms |
|---|---|---|---|
| G1 | ≥90 | | Normal or high |
| G2 | 60–89 | | Mildly decreased |
| G3a | 45–59 | | Mildly to moderately decreased |
| G3b | 30–44 | | Moderately to severely decreased |
| G4 | 15–29 | | Severely decreased |
| G5 | <15 | | Kidney failure |
| **Albuminuria category** | **AER (mg/24 h)*** | **ACR (mg/g)**** | **Terms** |
| A1 | <30 | <30 | Normal to mildly increased |
| A2 | 30–300 | 30–300 | Moderately increased |
| A3 | >300 | >300 | Severely increased |

*AER: Albumin excretion rate; **ACR: Albumin-to-creatinine ratio.

**Table 2:** Sources of error in GFR estimating based on serum creatinine [3,5].

| Source of error | Example |
|---|---|
| Non-steady state | • Rapidly changing renal function such as in acute kidney injury |
| Non-GFR determinants of serum creatinine | • (Race/ethnicity other than Caucasian or African American may be a factor) |
| Factors affecting creatinine generation | • Extremes of muscle mass<br>• Extremes of body size<br>• Diet and nutritional status<br>high protein diet<br>creatine supplements<br>vegetarian diet<br>• Muscle wasting diseases (e.g., paraplegia, amputations)<br>• Recent ingestion of cooked meat |
| Factors affecting tubular secretion of creatinine | • Decrease by drug-induced inhibition<br>trimethoprim<br>cimetidine<br>fenofibrate |
| Factors affecting extra-renal elimination of creatinine | • Dialysis<br>• Decrease by inhibition of gut creatininase by antibiotics<br>• Increased by large volume losses of extracellular fluid |
| Higher GFR | • Higher biological variability in non-GFR determinants relative to GFR<br>• Higher measurement error in serum creatinine and GFR |
| Interference with creatinine assay (method specific) | • Spectral interferences (e.g., bilirubin, some drugs)<br>• Chemical interferences (e.g., glucose, ketones, bilirubin, some drugs |
| Other patient groups | • Pregnancy<br>• Age below 18 years |

mated glomerular filtration rate, eGFR) simply from the serum creatinine result and some easily accessible demographic data. Evaluation according to albumin excretion needs albumin measurement in the patient urine. Another important and valuable test for CKD is Cystatin C; although, currently not commonly used, cystatin C is increasingly recognised.

Although these examinations are extremely important, they are analyzed by using different analytical methods and techniques and therefore different results can be obtained and reported with different units. This situation gives rise to confusion and difficulties of interpretation. For this reason, like many countries, standardization of assays and reporting in all our country, provides a great benefit for screening, diagnosis, staging and treatment follow-up of CKD.

The proposed roadmap for each test is given separately below. It is possible to achieve this standardization by simple arrangements or information. The same information is also important for in vitro diagnostic companies. In this respect diagnostic companies also should provide service compatible with laboratory practice.

## 2 Evaluation of CKD

GFR is a component of the excretory function of the kidney, but it is widely accepted as the best measure of kidney function. Because GFR is decreased due to structural kidney injury. Additionally, loss of other kidney functions is parallel to decrease of GFR.

GFR is detected by clearance of an endogenous or exogenous filtration marker. All clearance calculation methods with exogenous markers have some difficulties in clinical practice. For this reason, serum concentration of an endogenous marker are used most commonly for clearance calculations. **The main endogenous marker in routine use is creatinine**. Recently, serum cystatin C concentration has also been recognised as a valid alternative endogenous marker.

GFR estimation through serum creatinine concentration (eGFR) is valid for many clinical conditions of CKD such as diagnosis, staging, monitoring and detection of progression. When eGFR estimated through serum creatinine is not accurate due to error sources, detection of GFR by serum cystatin C concentration or creatinine clearance calculated using a 24 h urine collection in addition to a

**Table 3:** Standard reference materials for creatinine assays.

| Reference materials | Material form | Creatinine concentration | Manufacturer |
|---|---|---|---|
| SRM 914a | Crystallized creatinine | 99.7%±0.3 mass fraction | NIST |
| SRM 909b-1 | Lyophilized human serum | 56.18±0.55 µmol/L | NIST |
| SRM 909b-2 | Lyophilized human serum | 467.4±5.3 µmol/L | NIST |
| SRM 967-1 | Frozen human serum | 66.5±1.9 µmol/L | NIST |
| SRM 967-2 | Frozen human serum | 346.2±7.3 µmol/L | NIST |
| BCR-573 | Human serum | 68.7±1.4 µmol/L | IRMM |
| BCR-574 | Human serum | 105±1.3 µmol/L | IRMM |
| BCR-575 | Human serum | 404.1±7.1 µmol/L | IRMM |
| LN-24 | Fresh human serum | 44.3–354.9 µmol/L | CAP |

NIST: National Institute of Standards and Technology; IRMM: Institute for Reference Materials and Measurements; CAP: College of American Pathologists.

serum sample may be valuable. Sources of error in GFR estimating using creatinine are presented in Table 2.

## 2.1 Which creatinine measurement method?

In general, creatinine is measured by methods using the alkaline picrate (Jaffe) reaction in both our country and the World. But the Jaffe reaction is not specific for creatinine. Many compounds interfere with Jaffe method. Mainly proteins, glucose, ascorbic acid, creatine, ketone bodies, pyruvate, guanidine and cephalosporins [6]. Although some techniques have been developed to eliminate these interferents such as aluminium silicate absorption, acid blank use, ion exchange resine use or oxidation of interferents, these techniques have not been widespread used. Currently, almost all creatinine measurements are performed by automated instruments. Kinetic measurements within 20 to 80 seconds after starting of the reaction can highly decrease interference. Recently, as a new application, some diagnostic companies has developed "compensated" Jaffe method in which final creatinine results are automatically reported by reducing 0.20 to 0.30 mg/dL. Compensated methods give more compatible creatinine results with the reference method, isotope dilution mass spectrometry (IDMS) within the reference interval [7]. It is important to note that the creatinine assays from different manufacturers based on the Jaffe reaction are not all the same, and are likely to react in different ways to various interferences.

Among the routine creatinine measurement methods enzymatic method is the most compatible with reference method with less frequent interferences due to so called "non-creatinine chromogens" [7]. Although not as commonly used as Jaffe method, the enzymatic method is increasingly used due to its accuracy.

## 2.2 What should be the traceability of creatinine assays?

Since serum creatinine is used for estimation of GFR, different creatinine methods should be compatible with each other. Therefore, Certified Reference Materials (or Standard Reference Materials) in which creatinine concentrations were determined by reference measurement method (IDMS) have been developed by some authorities for the production of calibrators of diagnostic companies and harmonization among the commercial kits. Appropriate reference materials, methods and laboratories are listed on the database from the JCTLM (Joint Committee for Traceability in Laboratory Medicine, http://www.bipm.org/jctlm/). The commercial kits should be traceable to these reference materials. These reference materials may also be used for accuracy of clinical laboratories. A list of available standard reference materials is presented in Table 3.

## 2.3 Which formula should be used for eGFR by creatinine?

GFR is the best parameter reflecting kidney function with only single number. The "gold standard" is inuline clearence for GFR. Additionally, clearences based on **$^{51}$Cr-EDTA, $^{99m}$TC-DTPA, $^{125}$I-iotalamat**, and **Iohexol** are also precious as silver standard. However, all of these clearance tests are invasive, arduous, time consuming and expensive. Creatinine clearance and Cystatin C clarence accepted as "bronze standard" are widespread because of their characteristics such as easy performed, endogenous, cheapness and lack of time consuming. Currently, creatinine clearance is more widespread than cystatin C clearance.

Since both serum and urine concentrations of endogenous markers and timed (24 hours in general) urine

**Table 4:** CKD-EPI formulas for aduılts (results in mL/min/1.73m$^2$).

| Gender | Serum creatinine | CKD-EPI Formulas for estimating eGFR |
|---|---|---|
| Female | ≤0.7 mg/dL (≤62 µmol/L) | $144 \times (SCr/0.7)^{-0.329} \times 0.993^{Age}$ [if black x 1.159] |
| Female | >0.7 mg/dL (>62 µmol/L) | $144 \times (SCr/0.7)^{-1.209} \times 0.993^{Age}$ [if black x 1.159] |
| Male | ≤0.9 mg/dL (≤80 µmol/L) | $141 \times (SCr/0.9)^{-0.411} \times 0.993^{Age}$ [if black x 1.159] |
| Male | >0.9 mg/dL (>80 µmol/L) | $141 \times (SCr/0.9)^{-1.209} \times 0.993^{Age}$ [if black x 1.159] |

SCr: Serum creatinine concentration in mg/dL. If using µmol/L, mutiply results by 0.0113 to convert to mg/dL.

**Table 5:** Equations for eGFR according to serum cystatin C concentrations (results in mL/min/1.73m$^2$).

| | SCysC | eGFR formula |
|---|---|---|
| Female - Male | ≤0.8 mg/L | $133 \times (SCysC/0.8)^{-0.499} \times 0.996^{Age}$ [if female x 0.932] |
| Female - Male | >0.8 mg/L | $133 \times (SCysC/0.8)–^{1.328} \times 0.996^{Age}$ [if female x 0.932] |

SCysC: Serum cystatin C concentration (mg/L).

collection are required for clearance calculation, GFR estimation formulas have been developed through serum concentrations. There have been developed over 25 formulas using serum creatinine concentration and age, gender, race, and body mass for GFR estimation [8]. Until recently the most commonly used formulas among them are **Cockgroft – Gault (C-G)** and **Modification of Diet in Renal Disease (MDRD)** formulas. C-G formula is relatively more cumbersome because of the need for measurement of body weight. The original form of the MDRD formula (1999) requires age, gender, race (black and white),and serum concentrations of creatinine, urea and albumin as variables. This formula has been simplified by omitting variables of urea and albumin [9]. Since the Jaffe method gives higher values than IDMS, later the formula has been modified by changing the coefficient considering IDMS-traceable enzymatic method [10]. If the creatinine assay is not IDMS - traceable 186 is used as the coefficient and 175 is used as coefficient if the assay is IDMS-traceable. MDRD formula is superior to C-G formula, but it has also some limitations. Therefore The Kidney Disease Epidemiology Collaboration (CKD-EPI) formula was developed in 2009 and better results were obtained [11,12].

In the CKD-EPI formula, four different arrangements have been done according to gender and creatinine concentrations, above or below 0.7 mg/dL in women and 0.9 mg/dL in men, and a factor for blacks has been added (Table 4).

The CKD-EPI formula has been adopted worldwide and eGFR can be reported up to 90 mL/min/1.73 m$^2$. Whereas only <60 mL/min/1.73 m$^2$ eGFR values can be reported numerically with MDRD formula and higher values are reported as >60 mL/min/1.73 m$^2$. With the CKD-EPI formula, eGFR values >90 mL/min/1.73 m$^2$ can be reported numerically or as >90 mL/min/1.73 m$^2$. These options are left to the laboratory management. But >60 mL/min/1.73 m$^2$ GFR values can be safely reported by the CKD-EPI formula. **Therefore, when the eGFR is automatically reported through serum creatinine, the use of CKD-EPI formula is recommended**.

## 2.4 What should be the analytical performance of the creatinine measurement method?

Within subject biological variation of creatinine is lower or the individuality of creatinine is higher. Those error limits based on biological variation, 2.98% for imprecision and 3.96% for bias have been reported [13]. These limits can be rounded as 3% for imprecision and 4% for bias.

## 2.5 Is eGFR estimation by cystatin C useful?

Cystatin C, a low molecular weight protein (12.8 kDa), is a protease inhibitor synthesized in all nucleated cells. It is freely filtered through the glomeruli. The production rate is constant. Serum cystatin C concentration is not affected by diet, muscle mass and gender although steroids, obesity and thyroid dysfunction can influence serum concentrations. For this reason, it has been reported that cystatin C is a better surrogate marker of GFR than serum creatinine [3,12]. Especially when serum cretatinine may give an erroneous result, estimation of eGFR by cystatin C is recommended. Like serum creatinine, different equations depended on concentration are used for estimation

**Table 6:** Error sources for eGFR estimation by cystatin C [3].

| Source of error | Example |
|---|---|
| Non-steady state | • Acute kidney injury |
| Non-GFR determinants of serum cystatin C that differ from the study populations in which equations were developed | • Race/ethnicity other than US and European black and white |
| Factors affecting cystatin C generation | • Disorders of thyroid function<br>• Administration of corticosteroids<br>• Other hypothesized factors based on epidemiologic associations (diabetes, adiposity) |
| Factors affecting tubular reabosrption of cystatin C | Currently none |
| Factors affecting extra-renal elimination of cystatin C | Increased by severe decrease in GFR |
| Higher GFR | • Higher biological variability in non-GFR determinants relative to GFR<br>• Higher measurement error in cystatin C and GFR |
| Interference with cystatin C measurement | • Heterophilic antibodies |

of eGFR by cystatin C. The formulas used for eGFR by cystatin C are presented as following (Table 5).

## 2.6 What are the differences between cystatin C and creatinine?

Estimation of eGFR by cystatin C is superior to creatinine and confirmatory for CKD staging and prognosis either less than or over 60 mL/min/1.73 $m^2$ eGFR values [3]. However, cystatin C is much more expensive than creatinine. Moreover still there may be standardization problems. Recently a Standard Reference Material with human serum matrix (ERM-DA471/IFCC Cystatin C in human serum, 5.48 mg/L) has been prepared by IRMM and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Nevertheless, traceability problems may remain for commercial products. **Cystatin C measurements should be performed by traceable diagnostic products**.

## 2.7 Are there situations of erroneous eGFR result by cystatin C?

There are limitations also for eGFR estimation by cystatin C. These are presented in Table 6.

## 2.8 Can serum creatinine and cystatin C concentrations be combined for eGFR?

Recent literature data show that eGFR estimated through a combination of serum creatinine and cystatine C concentrations gives much more accurate results as compared to eGFR obtained by only serum creatinine or cystatin C, especially in the range of 45–59 mL/min/1.73 $m^2$ [14]. The equations derived from the combination of serum creatinine and cystatin C concentrations are presented in Table 7.

## 2.9 What should be done for pediatric patients?

The enzymatic creatinine assay should be preferred in pediatric patients. Height is also used for eGFR estimation by serum creatinine in children. eGFR should be used if the height of pediatric patient is known, but should not if the height is unknown. Serum urea nitrogen (BUN) is also used in a second equation [3,15]:

41.3 x (height/SCr)

$40.7 \times (\text{height}/\text{SCr})^{0.64} \times (30/\text{BUN})^{0.202}$

SCr: serum creatinine concentration (mg/dL); BUN: blood urea nitrogen (mg/dL); height: meter

$70.69 \times (\text{SCysC})^{-0.931}$

SCysC: serum cystatin C (mg/L)

## 2.10 How the results should be reported?

- When serum creatinine is being reported with conventional units (mg/dL), the number is rounded to the nearest 100$^{th}$ of a whole number, when expressed as SI unit (µmol/L) the number is rounded to the nearest whole number. For example, 1.25 mg/d Land 111 µmol/L (The conversion factor is 88.4 from conventional unit to SI unit).
- eGFR result based on creatinine is rounded to the nearest whole number. For example, 81 mL/min/1.73

**Table 7:** eGFR equations derived by the combination of serum creatinine and cystatin C [3]. Results in mL/min/1.73 $m^2$.

| Gender | Serum creatinine | Serum cystatin C | eGFR equations |
|---|---|---|---|
| Female | ≤0.7 mg/dL (≤62 µmol/L) | ≤0.8 mg/L | $130 \times (SCr/0.7)^{-0.248} \times SCysC/0.8)^{-0.375} \times 0.995^{Age}$ [if black x 1.08] |
| | | >0.8 mg/L | $130 \times (SCr/0.7)^{-0.248} \times SCysC/0.8)^{-0.711} \times 0.995^{Age}$ [if black x 1.08] |
| Female | >0.7 mg/dL (>62 µmol/L) | ≤0.8 mg/L | $130 \times (SCr/0.7)^{-0.601} \times SCysC/0.8)^{-0.375} \times 0.995^{Age}$ [if black x 1.08] |
| | | >0.8 mg/L | $130 \times (SCr/0.7)^{-0.601} \times SCysC/0.8)^{-0.711} \times 0.995^{Age}$ [if black x 1.08] |
| Male | ≤0.9 mg/dL (≤80 µmol/L) | ≤0.8 mg/L | $135 \times (SCr/0.9)^{-0.207} \times SCysC/0.8)^{-0.375} \times 0.995^{Age}$ [if black x 1.08] |
| | | >0.8 mg/L | $135 \times (SCr/0.9)^{-0.207} \times SCysC/0.8)^{-0.711} \times 0.995^{Age}$ [if black x 1.08] |
| Male | >0.9 mg/dL (≤80 µmol/L) | ≤0.8 mg/L | $135 \times (SCr/0.7)^{-0.601} \times SCysC/0.8)^{-0.375} \times 0.995^{Age}$ [if black x 1.08] |
| | | >0.8 mg/L | $135 \times (SCr/0.7)^{-0.601} \times SCysC/0.8)^{-0.711} \times 0.995^{Age}$ [if black x 1.08] |

SCr: Serum creatinine concentration; SCysC: Serum cystatin C concentration. Creatinine concentration in mg/dL. If using µmol/L, multiply results by 0.0113 to convert to mg/dL.

$m^2$ instead of 81.2 mL/min/1.73 $m^2$. When reporting serum cystatin concentration as conventional unit, the number is rounded to the nearest 100$^{th}$ of a whole number.

- When reporting eGFRCys and eGFRCreat-Cys, the number is rounded to the nearest whole number with the unit of mL/min/1.73 $m^2$.
- eGFR levels <60 mL/min/1.73 $m^2$ should be reported as "decreased".

# 3 Evaluation of Albuminuria and Proteinuria

Proteinuria may result from abnormal loss of plasma proteins due to [1] increased glomerular permeability to large molecular weight proteins (albuminuria and glomerular proteinuria), [2] disordered tubular reabsorption of normally filtered low molecular weight proteins (tubular proteinuria), [3] increased plasma concentration of low molecular weight proteins (overflow or overproduction proteinuria like immunoglobulin light chains). Proteinuria may also result from abnormal loss of proteins derived from the kidney (as constituents of renal tubular cells) or lower urinary tract. Albuminuria, tubular proteinuria or proteinuria derived from renal tubular constituents are indicators of kidney injury. Moreover, proteinuria is also important for progression of CKD.

Normally albumin is found in urine but albumin loss is increased in kidney injury. The primary urine protein is albumin in many kidney diseases. Recent epidemiologic data show that there is a strong relationship of the quantity of urine albumin with both kidney and cardiovascular disease risk [3]. Albuminuria is a common but a variable finding in CKD. Albuminuria is the earliest marker of glomerular disease, including glomerulosclerosis before GFR decrease. It is also an important marker in hypertensive nephrosclerosis but may not be detected before GFR decrease. Urinary albumin is increased in hypertension, obesity and vascular diseases.

## 3.1 What is the first step for evaluation of albuminuria? Is there any algorithm?

The early morning urine albumin measurement is initial test for proteinuria and it should be reported as albumin: creatinine ratio. If urine albumin cannot be measured, urine protein should be measured and reported as protein:creatinine ratio; when neither albumin nor protein can be measured, in descending order of preference, reagent strip urinalysis for total protein with automated reading and reagent strip urinalysis for total protein with manual reading are suggested [3].

## 3.2 Why measurement of urine albumin is more valuable than urine protein?

Urine albumin is more sensitive and specific than urine protein for glomerular damage. It reflects glomerular pathology in systemic diseases including diabetes, hypertension and systemic glomerulosclerosis [16]. Additionally, urine protein measurement has some technical problems. There is not only one protein in the urine and urine protein composition shows major changes from one specimen to another; ratio of inorganic ions are higher in urine and protein concentration in urine changes in a wide concentration range. All of these factors negatively affect the precision and accuracy of urinary total protein measurements. Moreover, the protein fractions of urine do not react with the reagents equally [17]. The lack of reference measurement procedure and reference materials for urine

**Table 8:** The relationship between albuminuria and proteinuria categories [3].

| | Categories | | |
|---|---|---|---|
| **Measure** | **Normal to mildly increased (A1)** | **Moderately increased (A2)** | **Severely increased (A3)** |
| AER (mg/24 hours) | <30 | 30–300 | >300 |
| PER (mg/24 hours) | <150 | 150–500 | >500 |
| ACR | | | |
| (mg/mmol) | <3 | 3–30 | >30 |
| (mg/g) | <30 | 30–300 | >300 |
| PCR | | | |
| (mg/mmol) | <15 | 15–50 | >50 |
| (mg/g) | <150 | 150–500 | >500 |
| Protein reagent strip | Negative to trace | Trace to + | + or greater |

AER: Albumin excretion rate; PER: Protein excretion rate; ACR: Albumin-to-creatinine ratio; PCR: Protein-to-creatinine ratio; A1, A2 and A3: categories of albuminuria/proteinuria.

protein is also an important problem in urine protein measurement and this is a difficult task.

## 3.3 Which method should be used for albumin?

Urine albumin should be measured by immunochemical methods which have high specificity for urine albumin, good precision and giving reliable quantitative results in lower albumin concentrations. Mostly, immunoturbidimetric assays are used in clinical laboratories for this aim today. There is not a reference measurement procedure and a reference material also for albumin yet. However, a joint committee of NKDEP and IFCC is working for standardization. Currently, the majority of commercially available kits uses calibrators traceable to CRM 470 (ERM-DA 470k/IFCC, 37.2 g/L), a reference material with serum matrix manufactured by IRMM.

## 3.4 How urine albumin should be reported?

Urine albumin should be reported as urine albumin-to-creatinine ratio (ACR). If conventional unit (mg/g) is used for ACR, the results should be expressed as whole numbers; if SI unit (mg/mmol) is used, the results should be expressed to one decimal place. Because of misleading, "microalbuminuria" or "macroalbuminuria" expressions should not be used anymore. The use of expressions "moderately increased albuminuria" for microalbuminuria and "severely increased" for macroalbuminuria are recommended. Additionally, protein measurements in untimed urine specimens should also be reported as the protein-to-creatinine ratio.

## 3.5 Why 30 mg/g threshold is recommended as upper reference limit of albuminuria for evaluation of CKD?

Albuminuria or proteinuria are generally expressed as urinary loss rate. Although there is not a real physiological excretion, the urinary loss of albumin and protein are expressed as **Albumin Excretion Rate (AER) or Protein Excretion Rate (PER)**. A threshold for ACR ≥30 mg/24 hours (or ≥3 mg/mmol) sustained for 3 months or more has been recommended to indicate CKD. This threshold is greater than three times the normal rate in young males and females (10 mg/ 24 hours) and approximately equivalent to an ACR of 30 mg/g (or 3 mg/mmol) in untimed urine. The relationship between AER, PER and albuminuria/proteinuria categories are presented in Table 8.

## 3.6 Which one should be preferred? 24 hours, untimed, early (first) morning, or second morning urine?

Reference point for albuminuria is based on the albumin concentration of the accurately collected timed 24-hour specimen. But the accurate collection of 24-hour urine is difficult in routine laboratory practice. This difficulty may contribute to errors in estimation of protein losses. Albuminuria estimation in untimed random urine has the sufficient discriminatory power to exclude proteinuria.

**Table 9:** Preanalytical and analytical factors affecting urine albumin/creatinine ratio [3].

| Factor | Example |
|---|---|
| **Preanalytical factors** | |
| Transient elevation in albuminuria | Menstrual blood contamination |
| | Symptomatic urinary tract infection |
| | Exercise |
| | Upright posture (orthostatic proteinuria) |
| | Other conditions, increasing vascular permeability (e.g., septicemia) |
| Intraindividual variability | Intrinsic biological variability |
| | Genetic variability |
| Preanalytical storage conditions | Degradation of albumin before analysis |
| Non-renal causes of variability in creatinine excretion | Age (lower in children and older people) |
| | Race (lower in Caucasian than black people) |
| | Muscle mass (e.g., lower in people with amputations, paraplegia, muscular dystrophy) |
| | Gender (lower in women) |
| Analytical factors | |
| Antigen excess (prozone) effect | Samples with very high albumin concentrations may be falsely reported as low or normal |

Therefore albumin-to-creatinine ratio in untimed urine is valid for albuminuria estimation. However, albuminuria positive results should be confirmed by albumin measurements in an early morning urine. Because early morning urine albumin is more compatible with 24-hour urine and has a lower biological variability. If there are unreliable causes for untimed urine albumin, such as increased biological variation, some pathological or physiological factors (Table 9), repeated albumin measurement in a timed specimen is recommended for confirmation.

### 3.7 Why is it necessary to rate albumin to creatinine?

Urinary concentrations of albumin or protein are changed proportionally to fluid intake by the patient. Therefore, different albumin and protein results can be obtained in concentrated or diluted urine specimens. Whereas creatinine excretion is constant all day long. Hence the albumin results should be reported as albumin-to-creatinine ratio.

### 3.8 What is the approach to pediatric patients?

There are not reference intervals for protein and albumin excretion encompassing all children. The results may be changed according to age, gender, pubertal status, obesity, exercise, fever, and posture. Because of the lack of maturation in the proximal tubular reabsorption, generally neonates and young infants/children are both expected to have higher urinary losses of both glomerular and tubular proteinuria. Therefore, by considering the affecting factors, adult proteinuria categories may be used for children older than 2 years. In general, albumin measurement is preferred, although some clinicians prefer total protein measurement. The current adult albuminuria categories cannot be applied in the children <2 years and adolescents with orthostatic proteinuria [3]. But proteinuria categories can be used instead of albuminuria.

## 4 Conclusions: Key Recommendations

The main tasks of laboratory specialists can be summarized as follows:

1. Creatinine assays should be traceable to a reference material which creatinine concentration assigned by GC-IDMS technique.
2. When reporting the creatinine result, eGFR should also be reported in adult (>18 years) population. A warning expression should be included in the report form if eGFR result is <60 mL/min/1.73 $m^2$.
3. eGFR values should be expressed quantitatively up to 90 mL/min/1.73 $m^2$ by CKD-EPI equation. Above 90 mL/min/1.73 $m^2$, eGFR values can be expressed quantitatively or >90 mL/min/1.73 $m^2$.
4. eGFR equations of the adult population should not be used for pediatric population. Different equations utilizing also patient height should be used. The enzymatic creatinine assay should be preferred. eGFR based on cystatin C can be used for confirmation in

the pediatric population.

5. Cystatin C measurements, at least when eGFR based on creatinine is not reliable and for confirmation should be encouraged.
6. Proteinuria or albuminuria values should be reported in proportion to creatinine.

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