AACC Guidance Document on Laboratory Investigation of Acute Kidney Injury

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Abstract

Background: Acute kidney injury (AKI) is a sudden episode of kidney damage or failure affecting up to 15% of hospitalized patients and is associated with serious short- and long-term complications, mortality and health care costs. Current practices to diagnose and stage AKI are variable and do not factor in our improved understanding of the biological and analytical variability of creatinine. In addition, the emergence of biomarkers, for example cystatin C and insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases 2 ([IGFBP7].[TIMP2]), and electronic notification tools for earlier detection of AKI, highlights the need for updated recommendations to address these developments.

Content: This AACC Academy guidance document is intended to provide laboratorians and clinicians up to date information regarding current best practices for the laboratory investigation of AKI. Topics covered include: clinical indications for further investigating potential AKI, analytical considerations for creatinine assays, the impact of biological variability on diagnostic thresholds, defining "baseline" creatinine, role of traditional markers (urine sodium, fractional excretion of sodium, fractional excretion of urea, and blood urea to creatinine ratio), urinary microscopic examination, new biomarkers, improving AKI-associated test utilization, and the utility of automated AKI alerts.

Summary: The last decade brought us a significant number of new studies characterizing the performance of existing and new biomarkers, as well as potential new tools for early detection and notification of AKI. This guidance document is intended to inform clinicians and laboratorians on the best practices for the laboratory investigation of AKI based on expert recommendations where the preponderance of evidence is available.

Footnote: Throughout this document, the term "blood" implies plasma or serum unless "whole blood" is explicitly stated.

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Introduction

Acute kidney injury (AKI) is a significant clinical complication affecting 10-15% of all hospitalizations and is defined as a rapid increase in blood creatinine and/or decrease in urine output.[1,2] While traditionally seen as a single disease or classified into renal-centric categories (i.e. prerenal, intrinsic, or postrenal AKI), AKI is now more specifically described as a syndrome that includes hepatorenal, cardiorenal, nephrotoxic, peri-operative and sepsis-associated AKI, among others.[3] Its co-existence with other severe syndromes like heart failure, liver failure and sepsis that cause significant morbidity and mortality themselves, may mask the significance of AKI on short- and long-term outcomes and make it challenging to diagnose and treat.[3] Recent literature provides strong evidence that AKI is independently associated with higher risk of cardiovascular events after hospital discharge[4,5], affects short- and long-term outcomes in liver failure patients[6], and is associated with higher 60-day mortality in patients with septic shock.[7] In addition, the 72-hour period immediately after AKI distinguishes the risk of important kidney-specific long-term outcomes, such as incident or progressive chronic kidney disease, long-term dialysis, or all-cause death.[8] Therefore, it is essential that clinicians are aware of the clinical presentation of AKI and that laboratorians are providing them with the right tools to aid in early diagnosis and staging.

Over the last decade, new biomarkers and electronic tools have emerged that can predict those at greater risk for developing AKI or identify the earliest changes seen in AKI. However, the efficacy of these new biomarkers and electronic tools have been challenged in human clinical trials. In addition, the lack of universal access to these new technologies is a significant barrier for their implementation. However, this has not prevented certain groups from adopting them into their guidelines, such as cardiac surgeons, and some medical centers from putting specific

biomarker-guided management protocols in place.[9,10] This has led to considerable disparities in the identification and management of AKI care around the globe, highlighting a greater need for more uniform best practices in testing for AKI.

The purpose of this AACC Academy guidance document is to provide expert opinion from a multidisciplinary group of nephrologists and laboratory scientists based on the preponderance of available evidence to guide clinicians and laboratorians in their laboratory investigations of AKI, with the ultimate goal of promoting best practices to improve healthcare and patient outcomes. It must be remembered however, that situations where AKI may develop are not addressed by measurements alone, but also by implementing active measures to prevent the development of AKI or limit its severity.

Initiating Clinical Evaluation of AKI

Laboratory testing of blood creatinine and bedside monitoring of urine output are recommended routinely for the detection of AKI in the inpatient setting and are the basis of current diagnostic and staging criteria (Table 1). These must therefore be measured in all situations when patients are at risk of either having or developing AKI. This includes a wide range of clinical presentations to the hospital such as trauma, volume depletion, sepsis and serious infection (Table 2). Since the symptoms of AKI may be non-specific and vary with the underlying cause, patients with unexplained edema, fatigue, shortness of breath, confusion, nausea, seizures or coma should be investigated. Patients are also at risk when there has been a significant change in their clinical course during hospitalization such as cardiac surgery, other significant procedures under anesthesia or the development of hypotension. Patients should also be monitored when they are receiving medications that can be nephrotoxic and also those drugs that require dose adjustment with changes in renal function. In addition, certain patient

populations are at particular risk for AKI because of underlying conditions or reduced renal function at baseline. The frequency and duration of monitoring should be individualized based on the clinical situation and degree of risk.[2]

In the outpatient setting, measurement of blood creatinine is commonly undertaken and should always be measured in patients at high risk for developing AKI or with primary renal disease, where urinary albumin-to-creatinine ratio is also useful. This also applies to patients with a history of a disorder that can cause end organ damage such as diabetes mellitus or systemic lupus erythematosus. Finally, blood creatinine should be measured to monitor renal function when patients are at risk of AKI from medications or an intercurrent illness.

Analytical Performance of Creatinine Assays

The two major types of creatinine assays routinely used in clinical laboratories today incorporate the Jaffe alkaline picrate or enzymatic methodologies.[11] Historically, interferences affecting the accuracy of the traditional Jaffe method (as much as 15-25% false elevation reported at physiological concentrations) led to the development of rate kinetic and rate-blank kinetic alkaline picrate methods to improve method specificity and reduce susceptibility to interfering substances; including various proteins, glucose, acetoacetate, and others.[12] Enzymatic methods have been shown to have fewer interferences than the Jaffe methods but reports have also shown a number of interferences still exist, including dopamine and bilirubin, although these can often be resolved by practical solutions.[13,14] Overall, enzymatic assays demonstrate improved analytical sensitivity and specificity in comparison with Jaffe assays.[15] The use of point-of-care (POC) methods for measurement of creatinine is also common, typically performing the measurement directly in whole blood. Performance differences between Jaffe, enzymatic and POC methods have been observed. Therefore, the impact of creatinine assay

performance on the ability to detect acute increases in blood creatinine for the identification of AKI has been assessed based on previously determined biological variability and total error goals, mainly established for estimated glomerular filtration rate (eGFR) calculation in the setting of chronic kidney disease (CKD).

Sources of analytical variability in creatinine methods include assay imprecision, calibration variability (both between methods and day-to-day variability within methods), and analytical interferences. As the definition and staging of AKI depends largely on changes in blood creatinine, precision and freedom from interferences can be seen as the most important parameters in this setting. A low between-method bias is vital if AKI is monitored using results from more than one laboratory. Proficiency testing (PT) data collected from the measurement of specimens commutable with fresh-frozen serum have been useful for determining the interlaboratory variability, including total imprecision and calibration consistency, of various assays to inform the total error observed across routine methods. Accuracy-based programs, those that employ real patient serum pools with supplementation of crystalline creatinine and have assigned values by way of isotope dilution mass spectrometry (IDMS) reference measurement procedures (RMP), are critical for the determination of the interlaboratory variability that is equivalent to, and informative of, what would be seen in real patient samples for clinical use.

These assays have, over time, been standardized to IDMS RMPs by way of standard reference materials (SRM) such as those developed in conjunction by the National Institute of Standards and Technology (NIST; SRM 909b-1 and -2) and the Institute for Reference Materials and Measurements (IRMM; BCR 573, 574, and 575) or other sources listed on the Joint Commission for Traceability in Laboratory Medicine (JCTLM) database. As such, reduction in

interlaboratory variability in blood creatinine determination has been achieved through the standardization efforts of the National Kidney Disease Education Program (NKDEP) Laboratory Working Group. This is evident through a number of external PT and calibration verification surveys.

Based on the College of American Pathologists (CAP) PT C-C 2019 survey, the majority of laboratories reported using the kinetic alkaline picrate method without rate blanking (46.5%, n=2332) or an enzymatic-based method (38.2%, n=1918). Fewer laboratories employ the rate-blank kinetic alkaline picrate (12.2%, n=610) or standard Jaffe (3.1%, n=155) methods. This survey also reported average method inter-laboratory coefficient-of-variation (%CV) for kinetic Jaffe, rate-blank kinetic Jaffe, and enzymatic assays, which were 3.8%, 4.1%, and 3.0% respectively, for five non-commutable PT samples ranging from 1.63 to 4.53 mg/dL (144 to 400 µmol/L) creatinine (as determined by the all method mean). The CAP LN24-B 2019, which utilizes accuracy-based grading criteria on commutable materials, demonstrate wider distribution of results for alkaline picrate Jaffe methods (non-blanked) as compared to enzymatic assays with an average %CV of 4.5% versus 2.9%, respectively. However, the variability reported by PT surveys represents inter-laboratory variability (including different manufacturers of both methods) and does not necessarily reflect intra-laboratory variability, which matters more for monitoring serial changes in a patient. In fact, Jaffe methods from some vendors may have comparable assay variability to enzymatic methods (%CV < 3.0%), as shown in a study performed using pooled patient samples prepared at 5 different concentrations and measured over 20 days.[16] Whilst the mainstay in many diverse clinical care practices, POC creatinine measurements represent only a fraction of creatinine assessments performed and are under-represented in external quality control assessments as most have been granted waived status in

the US, not requiring annual PT under the Clinical Laboratory Improvement Amendments (CLIA). However, the reported analytical imprecision of POC devices varies widely between 3.6% to 12.9% depending on manufacturer, which makes some unsuitable for the detection or monitoring of AKI.[17-19]

Method performance specifications for total error, imprecision, and bias can be derived from inter- and intra-individual biological variation of creatinine.[20] For AKI, assay imprecision is the most important variable and assays can be labeled as meeting "optimum", "desirable" or "minimum" imprecision goals based on their reported analytical variability and the analyte's biological variability. Optimum assays have intra-laboratory analytical variability (CV_A) < 0.25 of the intra-individual biological variability of the analyte (CV_I), while desirable assays have $CV_A < 0.50\ CV_I$, and minimum performance assays have $CV_A < 0.75\ CV_I$.[21] Based on a recent meta-analysis, the reported intra-individual biological variability of blood creatinine in healthy adults is 4.5% (4.4-5.7% CI).[22] This implies, to meet optimum, desirable and minimum performance specifications, assays should have CV_A < 1.3%, 2.3% and 3.4% for measurement of creatinine, respectively. Therefore, creatinine methods with intra-laboratory variability CV_A > 3.4% are not recommended for routine use in the clinical laboratory.

Biological Variability and Diagnostic Thresholds

The current diagnostic criteria used for AKI are based on a rise in blood creatinine (Table 1) and/or fall in urine output, as recommended by the 2012 Kidney Disease Improving Global Outcomes guidelines.[2] However, recent studies have linked these recommendations with high false-positive rates of AKI diagnoses.[23] This rate was higher for patients with CKD, where 30.5% of patients with true blood creatinine ≥1.5 mg/dL were misdiagnosed with AKI (defined by 0.3 mg/dL change) versus only 2% of patients with true blood creatinine <1.5 mg/dL. In

addition to high creatinine values, the effect was exacerbated with increased number of measurements and greater assay variability. Therefore, it is important to consider establishing new diagnostic criteria for creatinine-based detection of AKI that factor in the observed biological and analytical variability.

As mentioned earlier, the reported intra-individual biological variability of blood creatinine in healthy adults is ~4.5%. Intra-individual biological variability has also been shown to be minimally affected by sex, age, or time between samples, as demonstrated by a large study involving 9,817 paired creatinine results from adult patients seen by general practitioners.[24] In addition, studies involving CKD patients also show that CV_I is similar even with increasing creatinine concentrations.[25,26] This information can be combined with the analytical variability (CV_A) to determine the reference change value (RCV) of blood creatinine, the point at which a true change in biomarker (i.e. not due to the random variation is the result) in an individual can be detected when performing serial measurements.[27] Therefore, any change in creatinine from baseline that is less than the respective RCV may not be considered significant at a given probability level. It is calculated using the formula:

$$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$$

Where CV_A represents intra-laboratory analytical variability (varies by assay), CV_I represents intra-individual biological variability (4.5% for creatinine), and a unidirectional Z-score = 2.33 for 99% probability, regarded as a highly significant change. A unidirectional approach is recommended as only increases in blood creatinine are relevant for the identification of reduced GFR. Of note, different Z- scores for different probability levels and bidirectionality may be used in this calculation (ex. bidirectional Z-score = 1.96 for 95% probability, regarded as a significant bidirectional change). Both 95% and 99% unidirectional calculations are reported in

Table 3, but in this report, we decided to use a 99% probability unidirectional Z- score of 2.33, which yields higher specificity, to address the high false positive rate seen using current KDIGO criteria. If clinicians prefer to have higher sensitivity for detecting AKI, lower Z- scores with lower probabilities may be used, but this comes at the expense of specificity and increases the rate of false positives. As mentioned previously, the reported intra-laboratory analytical variabilities for enzymatic assays and Jaffe vary by concentration and are around 1.0% to 3.0%.[16] Taken together, this yields an RCV of 15% to 18% across the range of reported creatinine values. To simplify, for most US laboratories who use enzymatic and Jaffe methods, changes in creatinine from baseline less than ~0.20 mg/dL (18 µmol/L) or ~20% (whichever is greater) are within analytical and biological variability, and therefore should not be considered clinically significant for AKI alerts. This may explain why the current AKI definition by KDIGO is not as specific in patients with higher baseline values of creatinine (Figure 1). Therefore, clinicians and laboratorians should be aware of the current limitations of using current KDIGO definitions for AKI, which result in high false positive rates in patients with high creatinine concentrations. Instead, we recommend laboratories consult Table 3 to determine the RCV that is applicable to their method. We also propose the study of this new RCV: +0.20 mg/dL (18 µmol/L) when baseline blood creatinine <1.00 mg/dL (88 µmol/L) or +20% when baseline blood creatinine >1.00 mg/dL (88 µmol/L), as new thresholds for diagnosing AKI in future clinical trials. We suspect this will improve sensitivity for AKI detection in patients with low baseline creatinine values and specificity for patients with high baseline creatinine values (Figure 1). Similar recommendations (RCV of +0.50 mg/dL [45 µmol/L] or 25%) have already been adopted by international societies for the detection of contrast-induced AKI.[28] Importantly, laboratories

184 using methods with poorer CVs will require the use of a higher clinical cutoff for detection of
185 potential AKI (Table 3).

186 **Defining "Baseline" Creatinine**

187 Internationally agreed definitions of AKI are predicated on comparing an index blood
188 creatinine concentration with an earlier 'baseline' creatinine result. The Acute Kidney Injury
189 Network (AKIN) definition of AKI defined the baseline sample as one being available within 48
190 h of the index sample. The 2012 KDIGO criteria extended this so that a relative increase of
191 ≥50% compared to a sample within 7 days of the index sample could also satisfy the diagnosis
192 (Table 1).

193 The baseline creatinine value is assumed to reflect an individual's premorbid, usual renal
194 function (homeostatic set-point) and is compared against the index ('current') sample in the
195 detection and staging of AKI. Defining this baseline kidney function sample in the diagnosis of
196 AKI has been an area of active debate. No consensus exists on how to optimally determine
197 baseline kidney function when multiple preadmission creatinine measurements are available. The
198 discussion has been thoroughly reviewed by Thomas et al.[29]

199 The three main approaches to obtaining a baseline value are:

200 1. Using a measured creatinine value within 7 days of the current value
201 2. Using a measured creatinine value between 7 and 365 days before the current value from
202 all results within the time window.
203 3. By imputation when creatinine results are unavailable e.g. by back-calculating the
204 creatinine concentration from a standardized estimated GFR (e.g. 75 mL/min/1.73 m^2)
205 using the individuals age, gender and race, or by using the population mean creatinine.

206 There are advantages and disadvantages to all of these approaches.[29] For example, using a
207 creatinine value from the previous seven days might not represent the true baseline if AKI had
208 begun to evolve prior to this, causing an under-recognition of AKI. Conversely, creatinine
209 concentrations in acutely unwell individuals may be lower than the true baseline due to
210 decreased production in this situation, causing an under-recognition of AKI. Whilst there has
211 been extensive debate on which should be the preferred approach there is little evidence on
212 which to base a recommendation.[30]

213 The 2012 KDIGO guideline advocated use of the lowest creatinine concentration during
214 the current hospitalization as the baseline value, although also allowing creatinine concentrations
215 from a longer time period in an otherwise stable patient without progressive CKD when more
216 recent creatinine concentrations are unavailable.

217 The 2012 European Renal Best Practice Guidelines recommend using the first
218 documented blood creatinine value of the current episode as 'baseline', rather than historical
219 creatinine values or a calculated value based on a presumed glomerular filtration rate (GFR) of
220 75 mL/min. They acknowledge that this is an area of contention and indeed were concerned
221 about the different interpretations being applied to baseline creatinine. They cite Siew et al. who
222 demonstrated that the use of the value at admission in the episode under consideration was best
223 associated with mortality risk.[31] However, this study evaluated the use only of various single
224 creatinine values – use of a mean or median value from the previous 7 to 365 day's results was
225 not studied.

226 In a later study from Siew et al, designed to mimic clinical practice, clinical nephrologists
227 were asked to determine their best estimate of a patient's baseline creatinine concentration, based
228 on careful review of clinical information and laboratory records.[32] These values were then

compared to baseline creatinine values calculated using a variety of approaches, namely; (1) the mean outpatient value, (2) the most recent outpatient value, (3) the nadir outpatient value, and (4) the most recent inpatient or outpatient value. Three-time intervals were also chosen for study: 7–365, 7–730, and 1–730 days before admission. The authors concluded that the mean outpatient blood creatinine measured within a year of hospitalization most closely approximated the nephrologist-adjudicated baseline blood creatinine values. This approach has been widely used to determine the baseline creatinine value in AKI detection algorithms. It should be noted however that laboratory information systems may not discriminate between inpatient and outpatient results when determining the mean value. Consequently, this approach may not define the premorbid value in patients that have had acute hospital admissions.

In the UK, automated reporting of AKI alerts by National Health Service (NHS) laboratories was mandated in 2014. The algorithm uses two approaches to baseline assessment. In one, the current creatinine concentration (C1) is compared against the lowest creatinine result within the previous 7 days (RV1) to calculate a C1/RV1 ratio. Secondly, C1 is compared against the median of values from the previous 8 to 365 days (RV2) to calculate the C1/RV2 ratio. If either of these ratios exceeds 1.5 then an AKI alert is generated. If only one reference value is available (i.e. RV1 or RV2), then this is used to calculate an AKI score. If no values exist in the previous 365 days, the algorithm will not calculate an AKI score, but if the creatinine concentration on the index sample exceeds the reference range then an alert that this could be due to either AKI or CKD is generated.

Introduction of the AKI alerting system in the UK was accompanied by a dramatic reduction in the repeat testing interval for blood creatinine in patients from primary care: 5 days versus 55 days (stage 1); 2 days versus 38 days (stage 2); and 1 day versus 53 days (stage 3),

252 suggesting that the alerts act as an important prompt to clinical action).[33] There was also an
253 accompanying increase in hospitalization rates of patients receiving AKI alerts.
254 Although there is evidence to suggest that electronic AKI alerts increase identification of
255 AKI, there is little evidence to suggest that they improve survival or reduce the need for renal
256 replacement therapy.[33,34] Given this, evidence to suggest that any particular algorithm or
257 approach to baseline creatinine definition is superior to another in terms of clinical outcomes is
258 lacking.
259 **Role of Traditional Markers**
260 Traditional markers used in assessing kidney injury and function include urine sodium,
261 fractional excretion of sodium, fractional excretion of urea and the serum urea to creatinine
262 ratio.[35] These tests are not part of current definitions of AKI and so do not formally contribute to
263 assessing the presence or severity of AKI in a patient. The roles ascribed for these tests have
264 been to assist with identifying the underlying etiology of AKI, with greatest emphasis on
265 separating prerenal azotemia from acute tubular necrosis (ATN). This distinction has been
266 recognized as a vital step in selecting the appropriate management, as prerenal causes are often
267 treated with increased fluids, whereas with intrinsic renal damage, removal of body fluid may be
268 impaired and administering intravenous fluids may be contra-indicated.[36] In most cases, the
269 diagnosis of prerenal AKI is straightforward from the clinical history and physical examination,
270 and volume resuscitation can be given with impunity. Yet, in some circumstances, the distinction
271 between the prerenal state and ATN is less straightforward, as with patients who have already
272 received considerable volume and have not yet improved, those with evidence of volume
273 overload, and those at risk for complications from volume expansion (e.g. a history of cardiac

dysfunction or cirrhosis). In these patients, additional evidence can be useful either to support further fluid resuscitation or to help avoid excess fluid infusion.

While the traditional markers discussed in this section are commonly used, are frequently described in textbooks, and appear in many online pages and calculator websites, the evidence base for their use is rather low.[37,38] Here, we summarize the tests and assess further data available on their utility.

Urinary Sodium (Random)

A low random urine sodium concentration (below 10 or 20 mmol/L) in the setting of oliguric AKI is consistent with sodium avid state and the preserved ability to retain sodium from the urinary filtrate, as seen in prerenal AKI.[36] A value above 40 mmol/L suggests that the kidney cannot normally conserve sodium, often seen in intrinsic AKI.[39] Of note a urine reference interval for spot urine sodium is not useful in making this determination and may be confusing, as a urine sodium within the reference interval, may be found in patients with intrinsic AKI.

Fractional Excretion of Sodium

The fractional excretion of sodium (FENa) is used to improve the diagnostic performance of the urine sodium test in assessing the cause of AKI by standardizing it to creatinine excretion.[40] It is expressed as:

$$\text{FENa (\%)} = [(\text{urine sodium} \times \text{plasma creatinine}) / (\text{plasma sodium} \times \text{urine creatinine})] \times 100$$

FENa less than 1% is consistent with prerenal AKI and >2% is consistent with ATN. However, it is important to note that FENa is also <1% in healthy patients, so it is important to only use in the setting of increased blood creatinine.

The limitations of using FENa as a diagnostic tool are important to note. It has a poor area-under-the-curve (AUC) for separating prerenal from intrinsic AKI in septic AKI patients

297 (AUC = 0.59) when used alone.[41] This is not surprising considering that a finding of low urinary
298 sodium or low FENa is not diagnostic of the prerenal state because these values can be low in
299 individuals who do not have AKI in the setting of a low sodium diet or high urine volume. It can
300 also be low in other causes of AKI when the kidney is sodium avid as with glomerulonephritis,
301 hepatorenal syndrome, renal allograft rejection and contrast-induced AKI.[38,42] Similarly, the
302 finding of a high urine sodium or high FENa is not diagnostic of ATN because this can be
303 present in the setting of high sodium intake without AKI, in diuretic use and with resolution of
304 AKI or resolution of renal obstruction.[40] Based on the varying results in different clinical
305 contexts, the performance characteristics of these tests vary greatly in published studies and the
306 only gold standard for the diagnosis of prerenal azotemia is the rapid resolution of AKI with
307 restoration of volume.[38] Nevertheless, taken together and in clinical context, these urine indices
308 can be of use, particularly when partnered with information gleaned from other tests like urine
309 microscopy (see section on "Role of Urinary Microscopic Examination").

310 The diagnosis of hepatorenal syndrome (HRS) in the setting of cirrhosis is a clinical one
311 and often one of exclusion. However, FENa, holds unrecognized potential to assist with this
312 differential diagnosis. Due to the physiology of cirrhotic circulation, virtually patients with
313 advanced cirrhosis have chronic renal hypoperfusion and have a FENa <1%, even in the absence
314 of AKI. The degree of sodium avidity in advanced cirrhosis that even patients with ATN
315 typically have a FENa <1% and the test has thus historically been thought unhelpful in
316 distinguishing HRS from ATN.[43] However, in several studies the FENa in patients diagnosed
317 with HRS clustered tightly around 0.15% and in each case were significantly lower than those
318 for patients with ATN.[44,45] While the values for ATN varied across studies based on diagnostic
319 definitions, it appears that extremely low FENa (<0.2%) is in fact be clinically useful for

distinguishing HRS from ATN and has been now incorporated into international ascites club criteria.[46]

Fractional Excretion of Urea

The fractional excretion of urea (FEUr) has been proposed to separate prerenal AKI from ATN in patients receiving diuretics which can alter the urinary sodium and therefore affect both the urinary sodium and the FENa. It is expressed as:

FEUr (%) = [(urine urea × plasma creatinine) / (plasma urea × urine creatinine)] ×100

A FEUr <35% is consistent with prerenal AKI whereas values greater than 50% are consistent with loss of tubular function.

Studies evaluating the performance of FEUr have been variable in their findings. In patients with circulatory shock FEUr was shown to be preferred to FENa, and also not affected by diuretics, however in another critical care setting the FEUr was not found to be useful for separating transient from persistent AKI, or predicting future AKI.[47,48] More recently the test was found to have excellent performance in patients with cirrhosis.[49]

Blood Urea-to-Creatinine Ratio

Normally, urea is filtered and reabsorbed by the kidneys, whereas creatinine is filtered and actively secreted. Urea reabsorption is increased in the proximal tubule in the setting of volume depletion and the blood urea will increase out of proportion to the rise in creatinine. In fact, this is the origin of the term "prerenal azotemia" or nitrogen in the blood. Indeed, a high blood urea-to-creatinine ratio can be seen as the serum corollary of a low FEUr. When both urea nitrogen and creatinine are measured in mg/dL, a ratio of >20:1 is suggestive of the prerenal state, whereas if urea is measured in mmol/L and creatinine μmol/L, then a ratio of >0.081:1 (or rounded up to >0.1:1 for convenience) is suggestive of the prerenal state.[47]

As with other traditional urine markers, this ratio is also of limited value when used in isolation and best used in clinical context. The poor performance of this ratio has been observed in several large clinical trials.[50,51] This poor performance may be due to the wide range of other factors that may affect levels of urea, which can be increased by significant protein intake or a catabolic state, as with corticosteroid use, or lowered in the setting of severe liver disease or malnutrition. Similarly, blood creatinine can be reduced in patients with very low muscle mass or elevated from medications that block the urinary secretion of creatinine, oral creatine supplements or large protein intake.

In summary, there appears to be some clinical utility in the traditional markers discussed above, provided that they are not used in isolation and their limitations are well understood.

Role of Urinary Microscopic Examination

Urine analysis dates back to the 17th century and is one of the oldest and most commonly utilized tests for differential diagnosis of AKI.[52] In patients with prerenal azotemia, urine microscopy is usually bland or may feature an occasional hyaline cast or fine granular cast.[52,53] In patients with ATN, urine sediment analysis typically contains renal tubular epithelial cells, granular casts, and muddy brown or cellular casts.[52,53] Therefore, urine microscopy can help differentiate these two entities, along with the clinical context and supporting data.

Urine microscopy can also help with the diagnosis of less common causes of AKI. The presence of significant hematuria, pyuria without bacteriuria, and cellular casts is consistent with glomerulonephritis.[54] This is typically accompanied by proteinuria on the dipstick. Pyuria without bacteriuria and or white blood cell casts is suggestive of acute interstitial nephritis.[55]

It is recommended to use a urine sediment scoring system based on the number of granular casts and renal tubular epithelial cells (RTEC) as evidenced per high-power field in

order to differentially diagnose AKI (Table 4).[52] A score greater than or equal to 2 is an extremely strong predictor of ATN.[52] Assessment of this scoring system using the AKI diagnosis at discharge as the gold standard indicated that urine microscopy conducted on the day of nephrology consultation was highly predictive of ATN.[52] This system has been validated in other studies.[56-58] Additionally, the scoring system was found to be significantly associated with increased risk of worsening AKI, as defined by worsened Acute Kidney Injury Network (AKIN) stages of AKI, need for dialysis, or mortality from AKI.[59] This urinary sediment score can thus be utilized to first differentiate between ATN and prerenal azotemia and second to prognosticate the hospitalization course of AKI. Urine microscopy is widely and inexpensively available and its use for the differential diagnosis of AKI may assist the nephrology community clinically to provide clearer diagnoses and therapies for AKI patients. The main limitation to urine microscopy is that the automated systems are not sensitive for urinary casts and the microscopy has to be performed manually by trained personnel or physicians.[60,61]

Role of New Biomarkers

Blood creatinine has been used for the detection of changes in kidney function since the 1960s, but with a half-life of about 4 hours in healthy adults (8 hours when creatinine clearance is reduced by 50%) it is slow to react and can take 24 to 40 hours to increase in response to kidney injury.[62] Cystatin C has been proposed as a biomarker for earlier detection of changes in kidney function. However, most forms of AKI primarily involve injury in the renal tubular epithelium, not the glomerulus, so a decrease in GFR alone (as measured by creatinine or cystatin C) is not a sensitive or early indicator.[62] Over the last decade, several new AKI biomarkers have been approved for use in humans in different countries, but only measurement of urinary insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of

metalloproteinases 2 (TIMP2) is approved by the Food and Drug Administration (FDA) in the US for the assessment of risk for moderate or severe AKI.[63] The [IGFBP7].[TIMP2] test is also available in Europe, where urinary neutrophil gelatinase-associated lipocalin (NGAL) has also been CE-Marked (Conformité Européenne) since 2009. However, FDA-approval/clearance has not yet been granted for clinical use for NGAL. While there is a plethora of other markers currently being evaluated for their potential use in the setting of AKI,[64] we will focus our review and recommendations on the utility of markers that currently have FDA-approval for clinical use, namely cystatin C and IGFBP7/TIMP2.

Cystatin C

Cystatin C is a molecule that is constantly produced by all nucleated cells in the human body and an established marker for kidney function.[65] Its utility as a marker for early detection of AKI has been proposed, but results have been mixed.

In a prospective cohort study involving 1,150 high-risk adult cardiac surgery patients, called Translational Research Investigating Biomarker Endpoints for Acute Kidney Injury (TRIBE-AKI), cystatin C was less sensitive for AKI detection than creatinine.[66] However, cystatin C appeared to identify a subset of patients with AKI at higher risk for adverse outcomes. The prognostic value of cystatin C was also confirmed in a separate prospective, observational study involving 412 adults admitted to the Coronary Care Unit.[67] In that study, cystatin C was a strong predictor of AKI and 2-year mortality. Similar findings were reported for its use in patients admitted to the emergency department, where it did not show superior performance to creatinine in detecting AKI.[68]

On the other hand, the combination of cystatin C with creatinine was shown to be beneficial for risk stratification and prognosis in patients after contrast media exposure.[69]

Cystatin C was also shown to predict renal recovery earlier than creatinine among patients with AKI, potentially shortening hospital stays by 1-3 days and significantly reducing costs.[70] This can be explained by the rapid changes in muscle mass seen in hospitalized patients, which can greatly affect creatinine but not cystatin C. However, cystatin C failed to indicate recovery prior to creatinine in certain clinical groups receiving therapy that may affect nucleated cells (like those receiving chemotherapy or with evidence of bone marrow engraftment).[70] Taken together, this information suggests that cystatin C may be more useful in detecting recovery in patients hospitalized for more than a few days, when muscle wasting is accelerated and creatinine is heavily affected.

Analytically, a limited number of cystatin C assays have been recently standardized, but disappointingly results remain discordant between different analytical platforms.[71,72] As a result, measurement of cystatin C cannot be universally recommended due to poor standardization, the lack of availability from most vendors and high cost (in comparison with creatinine) worldwide.[73] To laboratories with access to the assay, the reported analytical and biological variability for cystatin C are around 2.0%[74] and 4.0%[75], respectively, which yields an RCV of ~16% (See section "Biological Variability and Diagnostic Thresholds" for calculation). This was confirmed by a 12 months follow-up study involving 1071 patients undergoing coronary angiography where a blood cystatin C increase greater than 15% was the optimal cutoff for detection of AKI.[69] Cystatin C may be useful to monitor instead of creatinine for AKI in patients with non-steady creatinine states, like rhabdomyolysis, where creatinine production varies greatly within 24-48 hours.

Urinary [IGFBP7].[TIMP2]

The first FDA-approved test for the assessment of risk for AKI is [TIMP2].[IGFBP7], currently marketed as Nephrocheck® (Astute Medical, San Diego, CA, *now part of bioMérieux, Lyon, France*). Both TIMP2 and IGFBP7 are cell-cycle regulators that can induce cell-cycle arrest, and are mainly produced by the distal and proximal tubules, respectively.[76] They were discovered in 2013 as part of a prospective, multicenter investigation using a cohort of critically ill adult patients, and subsequently validated in an independent cohort (Sapphire study) using a clinical assay and in comparison with existing markers of AKI.[77] The Sapphire validation study reported superior performance of urinary [TIMP2].[IGFBP7] (also referred to as AKIRisk^{TM}) with an area under the curve (AUC) of 0.80 for the development of AKI (stage 2 or 3) within 12 hours. It also demonstrated that urine [TIMP2].[IGFBP7] outperformed urine NGAL (AUC: 0.72), plasma cystatin C (AUC: 0.71), urine KIM-1 (AUC: 0.70), plasma NGAL (AUC: 0.69), urine IL-18 (AUC: 0.69), urine pi-GST (AUC: 0.61) and urine L-FABP (AUC: 0.61). In addition, the risk of AKI (stage 2 or 3 within 12 hours) and major adverse kidney events occurring within 30 days increased when urinary [TIMP2].[IGFBP7] was above 0.3 $(ng/mL)^2/1000$, and drastically increased when value was above 2.0 $(ng/mL)^2/1000$. However, it is important to note the significant overlap between measured urinary [TIMP2].[IGFBP7] in healthy urine donors and the 0.3 threshold. This was also separately demonstrated by a large multi-center study that recruited 750 healthy subjects and chronic comorbid subjects without AKI, and where a reference interval of 0.04 – 2.22 $(ng/mL)^2/1000$ was established for urinary [TIMP2].[IGFBP7].[78] This overlap explains why the reported sensitivity and specificity for this test using a >0.3 $(ng/mL)^2/1000$ threshold is 92% and 46%, respectively, while using the higher threshold of >2.0 $(ng/mL)^2/1000$ yields 46% and 95%, respectively.[79] As a result, using a 0.3 $(ng/mL)^2/1000$ threshold provides better sensitivity but can yield a significantly high number of

false positives (~50% of healthy patients tested). It is possible that normalizing to urine creatinine or urine osmolality may improve the performance of this test, as demonstrated by a recent report that recruited healthy volunteers and measured urinary [TIMP2].[IGFBP7] before and after hydration, and showed a significant drop in their score.[80,81] However, data on biological variability of urinary [TIMP2].[IGFBP7] is lacking in the literature, and the effects of normalizing to urine creatinine or osmolality should be checked in critically ill patients at risk of AKI as well before it can be recommended for implementation.

The clinical performance of urinary [TIMP2].[IGFBP7] was also validated in the Opal[82] and Topaz[79] studies and tested in critically-ill patients with different etiologies.[83] So far, urinary [TIMP2].[IGFBP7] has been shown to provide early detection and risk stratification for imminent stage 2/3 AKI in over 1,800 critically-ill adult patients with different etiologies.[83] It is important to note the variable performance of the marker in studies using different cutoffs and timepoints. In several of the studies listed, there is significant deterioration in the performance of the marker when measured beyond 12 hours from an AKI event. In addition, it is not surprising that the AUC is lower in studies that attempted to use [TIMP2].[IGFBP7] to also detect AKI Stage 1, which it does not distinguish from healthy individuals as well and has not received FDA-approval for. However, clinical outcomes studies conducted by Meersch et al.[84] and Gocze et al.[85] both showed no significant difference between the intervention (i.e. use of [TIMP2].[IGFBP7]) and the control arms for the need of renal replacement therapy and mortality, and major adverse kidney events by 30 days.

In pediatric populations, fewer studies have been conducted but the markers are also showing promise (Table 5). However, a comprehensive approach that uses age-specific reference

intervals derived from pediatric patients is needed before [TIMP2].[IGFBP7] can be recommended in this population.[86]

Based on the current body of literature, urinary [TIMP2].[IGFBP7] is not yet recommended for routine risk assessment of AKI due to the lack of evidence of benefit shown in outcome studies, its suboptimal specificity at the recommended 0.3 $(ng/mL)^2/1000$ cutoff (causing a 50% false positive rate) and limited performance studies outside of the ICU or perioperative setting. This recommendation is consistent with the National Institute of Health and Care Excellence, based on the evidence reviewed as of June 17th, 2020.[87] Urinary [TIMP2].[IGFBP7] may play a role in specific populations, like perioperative care in cardiac surgery, when combined with other clinical and diagnostic findings, as an aid in the risk assessment for the development of moderate or severe (KDIGO Stage 2 or 3) AKI in patients ≥ 21 years who are at high risk for AKI. However, positive outcome studies and further optimization of different cutoffs and collection times for these specific populations are also needed prior to implementation.

For laboratories implementing this test for translational research or ultimately clinical purposes, they should verify that the reported reference interval of 0.04 – 2.22 $(ng/mL)^2/1000$ applies to their own population (using n=20 urine samples with 90% of samples within proposed range for acceptance) or otherwise should consider validating their own reference intervals using samples from healthy individuals (n=120), notwithstanding the substantial cost of such a validation. In addition, we recommend that the result report for [TIMP2].[IGFBP7] includes a clarifying statement to aid in interpreting results, like "Risk for developing moderate to severe AKI within 12 hours is low (AKIRisk ≤ 0.30 $(ng/mL)^2/1000$), moderate (AKIRisk = 0.31 – 2.00 $(ng/mL)^2/1000$), or high (AKIRisk > 2.00 $(ng/mL)^2/1000$)". We do not currently recommend

testing urinary [TIMP2].[IGFBP7] on patients < 21 years old, on those who are low risk for AKI such as ambulatory patients or those who had minor surgery, or performing daily or serial measurements of the markers. Finally, there is currently only one assay (Nephrocheck®) on which all of these studies have been conducted, therefore the derived thresholds and recommendations may not be applicable to a new assay for urinary [TIMP2].[IGFBP7], unless concordance with Nephrocheck® is clearly demonstrated.

Eliminating Wasteful Testing

Urine Eosinophils in Acute Interstitial Nephritis

The test for urine eosinophils is not useful to confirm or exclude acute interstitial nephritis and should no longer be considered in the evaluation of AKI.[88]

Utility of Automated AKI Alerts

The international consensus definition of AKI as defined by the KDIGO consortium is relatively straightforward. AKI is diagnosed when there is a 0.30 mg/dL (26.5 µmol/L) increase in creatinine within a 48 hour period or a 50% increase over 7 days.[2] There are urine output criteria as well, but detecting AKI based on urine output is beyond the reach of most clinical laboratories. The seemingly simple AKI definition requires only time- and individual-stamped creatinine values to be evaluated, but there are several complexities that need to be considered by the clinical laboratory.

First, the definition of AKI depends exquisitely on the creatinine value taken to be "baseline", which is not as straightforward to define (See section "Defining "Baseline" Creatinine"). Second, the 0.30 mg/dL (26.5 µmol/L) increase criterion increases the risk of false-positive AKI diagnoses in patients with CKD, while increasing the risk of false-negative AKI diagnoses in non-CKD patients with low creatinine values (Figure 1). Therefore, it is no surprise

that the data on notification of providers has been relatively mixed. To date, the only published randomized trial was a single-center study of 2,393 patients with AKI detected by an automated sniffer algorithm.[89] Randomization, at the patient level, to the alert group was not associated with clinical improvement (change in creatinine, dialysis, or death). However, there is some evidence to suggest that an AKI alert system, coupled to an educational program about AKI management, may have beneficial results. In a 5-center, stepped-wedge trial, an AKI alert coupled to an educational program decreased hospital length of stay and improved the rate of certain key best practice metrics.[90] However there was no difference in 30-day mortality.

Recently, researchers leveraged the US Veterans Affairs clinical database to create a data set of more than 700,000 individuals across 1,239 health care facilities and implemented a machine learning (ML)-based approach to predict AKI with great success (AUC = 0.92).[91] This AUC significantly exceeded that of other studies using novel blood and urine biomarkers to predict AKI, which rarely exceeds 0.75-0.80 (See section "Role of New Biomarkers"). However, there are major barriers to implementing ML. Most notably, the inclusion of a high number of variables as inputs (620,000 in this study), which can easily "break" if any single variable is changed (like when a lab information system is updated).[92]

Taken together, the value of communicating results regarding the presence of AKI is unclear. Whilst there is evidence of improved clinical practice, as yet this has not been linked to improved outcomes. If providers are to be informed, it is likely important to include a robust educational program to aid in their decision-making. Future studies to determine which subsets of patients and providers may benefit from alerts are necessary. Current definitions of AKI and "baseline" creatinine may also be contributing factors that should be investigated further.

Machine learning may hold a greater promise for accurate prediction of AKI but robust validation and continuous monitoring of these models will be essential to their success.[93]

Summary

Our understanding of and tools used for detecting AKI have both evolved since KDIGO was published in 2012. The information and opinions provided within this document are intended to shed light on the current status of the field and generate a healthy debate with clinical organizations that leads to a much-needed update to our current practice of investigating AKI. A summary of our findings and recommendations to laboratories and clinicians can be found in Table 6. Clinicians and laboratorians should work together to implement them, and researchers are needed to fill in the remaining gaps in our understanding of these testing strategies.

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795 **Tables**

796 Table 1: AKI Definition based on KDIGO 2012

Diagnostic criteria for AKI	
• Increase in blood creatinine by ≥ 0.3 mg/dL (26.5 µmol/L) within 48 hrs; or • Increase in blood creatinine to ≥ 1.5 times baseline, known or presumed to have occurred in the past 7 days; or • Urine volume <0.5 mL/kg/h for 6 hours	
AKI Staging	
AKI Stage I	• Increase in blood creatinine ≥ 0.3 mg/dL (26.5 µmol/L); or • Increase in blood creatinine to 1.5-1.9 times from baseline; or • Urine volume <0.5 mL/kg/h for 6-12 hours
AKI Stage II	• Increase in blood creatinine to 2.0-2.9 times from baseline; or • Urine volume <0.5 mL/kg/h for ≥12 hours
AKI Stage III	• Increase in blood creatinine to ≥ 3.0 from baseline; or • Blood creatinine ≥ 4.0 mg/dL (≥ 354 µmol/L); or • Initiation of renal replacement therapy; or • Decrease in eGFR to <35 mL/min/1.73m2 in patients <18 years; or • Urine volume <0.3 mL/kg/h for ≥24 hours; or • Anuria for ≥12 hours

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801 Table 2: Clinical scenarios that would require patient monitoring for development of AKI.

Clinical Scenario	**Examples**
Initial presentation	Volume depletion, trauma, sepsis, rhabdomyolysis, hypotension
Change in clinical course	Surgery (especially cardiopulmonary bypass), hypotension
Nephrotoxic medications	Aminoglycosides, vancomycin, radioiodine contrast, NSAIDs, chemotherapy
Susceptibility	Advanced chronic kidney disease (stage 3 or higher), diabetes mellitus, plasma cell dyscrasia, advanced liver disease, advanced cardiac disease

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803 Table 3: Relationship between analytical coefficient of variation (CV) and relative change value
804 (RCV) for creatinine. RCV was calculated using 4.5% within-subject biological variation, and
805 for a 95% probability unidirectional change and a 99% probability bidirectional change. As the
806 inputs to the equation and the physiology under examination are not precisely defined the outputs
807 should be considered as approximations only.

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Analytical CV (%)	95% Probability RCV (%)*	99% Probability RCV (%)**
0.1	11	15
1.0	11	15
2.0	12	16
3.0	13	18
4.0	14	20
5.0	16	22
6.0	18	25
7.0	19	27
8.0	21	30
9.0	24	33

10.0	26	36
11.0	28	39
12.0	30	42
13.0	32	45
14.0	34	49
15.0	37	52

*Z = 1.65

**Z = 2.33

Table 4: Urine microscopy scoring table for differential diagnosis of AKI. Score greater than 2 is a strong predictor of acute tubular necrosis.

Score	**Description**
1	RTE cells 0 and granular casts 0
2	RTE cells 0 and granular casts 1 to 5 *or* RTE cells 1 to 5 and granular casts 0
3	RTE cells 1 to 5 and granular casts 1 to 5 *or* RTE cells 0 and granular casts 6 to 10 *or* RTE cells 6 to 20 and granular casts 0

RTE: Renal tubular epithelial

820 Table 5: Summary of clinical trials involving [TIMP2].[IGFBP7] in pediatric populations.

The cause of AKI	Study	Patient population	AKI diagnostic criteria	AKI threshold	No. of patients enrolled/no. of patients developed AKI	[TIMP2].[IGFBP7] detection time	AUC	Cut off
Liver transplantation	Fuhrman et al. [94]	Patients (<18 years) undergoing liver transplantation	KDIGO	AKI within 48 - 96 h	16/6	At 6 h after liver transplant	0.93	NR
Cardiopulmonary bypass surgery	Dong et al. [95]	Patients (<18 years) undergoing cardiopulmonary bypass surgery	KDIGO	AKI within 72 h from surgery	150/50	At 2, 6, 12, and 24 h after cardiopulmonary bypass	0.83 (12 h)	NR
	Meersch et al. [96]	Patients (<18 years) undergoing cardiopulmonary bypass surgery	pRIFLE	AKI within 72 h from surgery	51/12	4 h after cardiopulmonary bypass	0.85	0.7
	Gist et al. [97]	Patients (<18 years) undergoing cardiopulmonary bypass surgery	KDIGO	AKI stage ≥ 1 within 72 h from surgery	94/31	At 2, 6, 12, 24, 48 and 72 h after cardiopulmonary bypass	0.71 (12 h alone) 0.79 (12 h with clinical model)	0.78
General	Westhoff et al. [98]	Patients (<18 years) referred to clinic with established AKI	pRIFLE	NR (30 d and 3 mo mortality)	133/46	At admission	0.84 (30 d mortality) 0.88 (3 mo mortality) 0.77 (renal replacement therapy)	0.3

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823 Table 6: Summary of findings and recommendations to laboratories and clinicians.

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#	Finding(s) and/or recommendation(s)	Target Group	
		Laboratory	Clinician
1	Monitor blood creatinine and/or urine output routinely for patients at risk of having or developing AKI. Frequency of length of monitoring should be individualized based on the clinical situation and degree of risk.		X
2	Only employ creatinine assays with intra-laboratory analytical variability ≤ 3.4% for detection of AKI.	X	
3	Evaluate the use of +0.20 mg/dL (18 µmol/L) or +20% (whichever is greater), as	X	X

	new thresholds for diagnosing AKI in future clinical trials.		
4	Laboratories measuring creatinine with analytical methods that have poor precision (CV_A > 3.4%) require the use of a higher clinical cutoff for diagnosis of AKI (refer to Table 3).	X	X
5	There is currently no evidence to suggest that any particular algorithm or approach to baseline creatinine definition is superior to another in terms of clinical outcomes.	X	X
6	A urinary sodium (random) test can be useful in distinguishing pre-renal (sodium < 20 mmol/L) from intrinsic AKI (sodium >40 mmol/L).		X
7	The fractional excretion of sodium (FENa) is used to improve the diagnostic performance of the urine sodium test in assessing the cause of AKI by standardizing it to creatinine excretion.		X
8	A fractional excretion of urea (FEUr) test can separate prerenal AKI from acute tubular necrosis and is preferred in the setting of diuretic use.		X

9	A blood urea-to-creatinine ratio of >20:1 is suggestive of the prerenal state, whereas if urea is measured in mmol/L and creatinine μmol/L, then a ratio of >0.081:1 (or rounded up to >0.1:1 for convenience) is suggestive of the prerenal state. This ratio has several limitations and performed poorly in large clinical trials.		X
10	Urine microscopy can help differentiate prerenal azotemia from acute tubular necrosis. It can also help with the diagnosis of less common causes of AKI, like glomerulonephritis and acute interstitial nephritis.		X
11	The use of a urine sediment scoring system based on the number of granular casts and renal tubular epithelial cells (RTEC) as evidenced per high-power field in order to differentially diagnose AKI is recommended (Table 4).		X
12	Cystatin C may be helpful in predicting renal recovery earlier than creatinine among hospitalized patients with AKI. However, the assay cannot be universally recommended due to poor standardization, the lack of	X	

	availability from most vendors and high cost (in comparison with creatinine) worldwide.		
13	Urinary [IGFBP7].[TIMP2] is not yet recommended for routine risk assessment of AKI due to the lack of evidence of benefit shown in outcome studies, high false positive rate and limited performance studies outside of the intensive care unit or perioperative setting.	X	X
14	The test for urine eosinophils is not useful to confirm or exclude acute interstitial nephritis and should no longer be considered in the evaluation of AKI	X	X
15	The value of automated alerts and communicating results regarding the presence of AKI is unclear. Whilst there is evidence of improved clinical practice, as yet this has not been linked to improved outcomes.	X	X

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827 **Figures**

828 Figure 1: Depicting the difference between using criteria outlined in this document (AACC-AKI, grey line, using 0.20 mg/dL [18

829 µmol/L] or 20%, whichever is greater) for detecting significant change in creatinine from baseline when compared with KDIGO 2012

830 criteria (using 0.30 mg/dL [26.5 µmol/L] criterion, yellow line). BCr: Blood Creatinine in mg/dL

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