Diseases Detected and Conditions Screened by Quantitative Urinalysis Using AutoUA®

Renal Diseases.

1. Glomerular Disorders.
   a. Glomerulonephritis.
   b. Nephrotic Syndrome.

2. Tubular Disorders.
   a. Acute Tubular Necrosis.
   b. Hereditary and Metabolic Tubular Disorders.

3. Interstitial Disorders.
   a. Acute Pyelonephritis.
   b. Chronic Pyelonephritis.
   c. Acute Interstitial Nephritis.

4. Renal Failure.
   a. Chronic Kidney Disease (CKD).
   b. Diabetic Kidney Disease (DKD).

5. Renal Lithiasis.

Urine Screening for Metabolic Disorders.

- *Overflow vs. Renal Disorders.*
- *Newborn Screening Tests.*
- *Amino Acid Disorders.*
- *Porphyrin Disorders.*
- *Mucopolysaccharide Disorders.*
- *Purine Disorders.*
- *Carbohydrate Disorders.*

Diseases Detected and/or Indicated by Quantitative Urine Assays Using AutoUA®

Disorders and Indicators of Normal Kidney Function Detected Using AutoUA® Quantitative Assay: pH.

1. Acidic Urine.

- Emphysema.
- Diabetes mellitus.
- Starvation.
- Dehydration.
- Diarrhea.
- Presence of acid-producing bacteria.
- High protein diet.
- Medications.

2. Alkaline Urine.

- Hyperventilation.
- Vomiting.
- Renal tubular acidosis.
- Urease producing bacteria.
- Vegetarian diet.
- Old urine samples.

Diseases Detected Using AutoUA® Quantitative Assays: Protein, Total Protein (micro-), Albumin (micro-), and NAP (Non-Albumin Protein, micro-).

- Kidney disease.
- Kidney infection.
- Kidney failure.
- Urinary tract obstruction such as kidney stones.
- Late-stage muscular dystrophy.
- Myasthenia gravis.
- Type 2 diabetes mellitus without complications.
- Essential (Primary) hypertension.
- Type 2 diabetes mellitus with hyperglycemia.
- Chronic Kidney Disease (CKD), stage 3 unspecified.
- Vitamin D deficiency, unspecified.
- Chronic Kidney Disease (CKD), stage 4 (severe).
- Hyperlipidemia, unspecified
- Type 2 diabetes mellitus with Diabetic Chronic Kidney Disease (DKD).
- Type 2 diabetes mellitus with other specified complication.
- Kidney transplant status.
- Kidney failure.
- Urinary tract obstruction such as kidney stones.

Diseases Detected Using AutoUA® Quantitative Assays: Protein, Total Protein (micro-), Albumin (micro-), and NAP (Non-Albumin Protein, micro-) Continues.

- Late-stage muscular dystrophy.
- Myasthenia gravis.
- Type 2 diabetes mellitus without complications.
- Essential (primary) hypertension.
- Type 2 diabetes mellitus with hyperglycemia.
- Chronic Kidney Disease (CKD), stage 3 unspecified.
- Vitamin D deficiency, unspecified.
- Chronic Kidney Disease (CKD), stage 4 (severe).
- Hyperlipidemia, unspecified.
- Type 2 diabetes mellitus with diabetic chronic kidney disease.
- Type 2 diabetes mellitus with other specified complication.
- Kidney transplant status.

Diseases Detected Using AutoUA® Quantitative Assay: Glucose.

- Gestational diabetes.
- Obesity.
- Type 2 diabetes.
- Hormonal dysfunction.
- Pancreatitis.
- Acromegaly.
- Cushing syndrome.
- Hyperthyroidism.
- Pheochromocytoma.
- Thyroxine.
- Growth hormone.
- Severe stress.
- Cerebrovascular trauma.
- Myocardial infarction.
- End-stage renal disease.
- Cystinosis.
- Fanconi syndrome.

Diseases Detected Using AutoUA® Quantitative Assays: Ketones, Total Ketones, Acetoacetic Acid, and β-hydroxybutyric Acid.

- Diabetes (Type 1) mellitus.
- Diabetic Keto Acidosis (DKA).
- Alcoholic Ketoacidosis.
- Severe hypoxia.
- End-stage renal disease.
- Hepatic ischemia.
- Metabolic disorders.

Diseases Detected Using AutoUA® Quantitative Assays: Ketones, Total Ketones, Acetoacetic Acid, and β-hydroxybutyric Acid Continues.

- Organ failure.
- Illness preventing adequate intake or absorption of carbohydrates.
- Accelerated loss of carbohydrates due to vomiting.
- Weight loss, eating disorders clinics, and strenuous exercise.

Diseases Detected Using AutoUA® Quantitative Assay: Blood (Hemoglobin).

- Disorders of renal or genitourinary origin and/or trauma or damage to the organs of these systems.
- Renal calculi.
- Glomerular diseases.
- Tumors.
- Trauma.
- Pyelonephritis.
- Exposure to toxic chemicals.
- Anticoagulant therapy.
- Intravascular hemolysis.
- Rhabdomyolysis.

Diseases Detected Using AutoUA® Quantitative Assay: Bilirubin.

- Bile duct obstruction (post-hepatic jaundice).
- Gallstones.
- Cancer.
- Liver damage (hepatic jaundice).
- Hepatitis.
- Cirrhosis.

Diseases Detected Using AutoUA® Quantitative Assay: Urobilinogen.

- Obstruction of the bile duct.
- Liver disease.
- Liver damage, disorders.
- Hepatitis.
- Cirrhosis.
- Carcinoma.
- Hemolytic disease/disorders.

Diseases Detected Using AutoUA® Quantitative Assay: Nitrite.

- UTI.
- Presence of bacteria.
- Cystitis.
- Pyelonephritis.
- Evaluation of antibiotic therapy.

Diseases Detected Using AutoUA® Quantitative Assay: Nitrite Continued.

- Monitoring patients at high-risk of UTI.
- Screening for urine cultures.

Diseases Detected Using AutoUA® Quantitative Assay: Leukocyte Esterase (wbcs).

- UTI.
- Bacterial and nonbacterial urinary tract infections.
- Inflammation of the urinary tract.
- Screening for urine culture specimens.

Diseases Detected Using AutoUA® Quantitative Assay: Specific Gravity.

- Patient hydration.
- Patient dehydration.
- Loss of renal tubular concentration ability.
- Ability of kidneys to concentrate the glomerular filtrate.
- Diabetes insipidus.
- Inadequate donor sample.

TEST DESCRIPTIONS

Renal Function Test.

a. GFR (eGFR) – Tubular reabsorption test.
b. Tubular secretion and renal blood flow tests.

Normalization.

a. Creatinine and Creatinine Normal Population Tables (age, sex).
b. See Normalization white paper.
c. Specific Gravity – qualitative at best measure of urine conc.
d. Creatinine – true urine concentration – used for normalization.

Physical Examination of Urine.

Color – Normal or Abnormal.
Clarity (nonpathological and pathological turbidity).

Chemical Test of Urine.

Types:

a. Qualitative – very poor substitute for quantitative chemistry.
b. Quantitative.

pH:

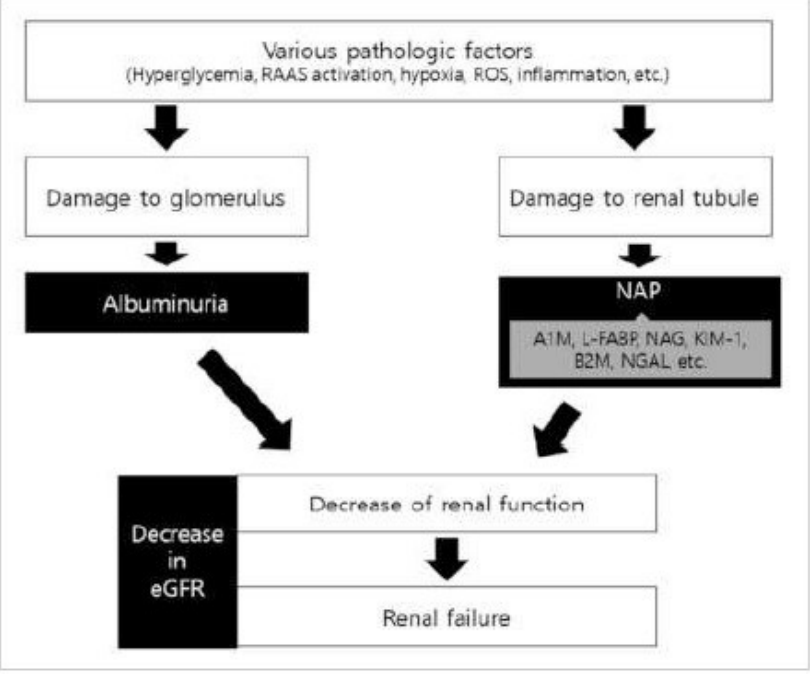
Clinical Significance: Indicator of normal kidney function. The kidneys are the major regulator of acid-base content in the body.

Protein:

a. Protein test in urine for the detection of **PROTEINURIA** to include NAP (non-albumin proteinuria and albuminuria). – Don't sleep on NAP!

i. Diabetic kidney disease (DKD) is one of the most common forms of chronic kidney disease. Its pathogenic mechanism is complex, and it can affect entire structures of the kidney. However, conventional approaches to early stage DKD have focused on changes to the glomerulus. Current standard screening tolls for DKD, albuminuria, and estimated glomerular filtration rate are insufficient to reflect early tubular injury. Therefore, many tubular biomarkers have been suggested. Non-albumin proteinuria (NAP) contains a wide range of tubular biomarkers and is convenient to measure.[3]

ii. The predictive power of NAPCR to DKD progression remained statistically significant in patients with normal-albuminuria or eGFR ≥ 60 ml/min/1.73 m^2, suggesting that NAPCR has a prognostic value for early stage DKD.



b. Albuminuria is well-known. It is important to monitor in case of potential development of Chronic Kidney Disease (CKD).

i. The development of diabetic nephropathy leading to reduced glomerular filtration and eventual renal failure is a common occurrence in people with both Type 1 and Type 2 diabetes mellitus. Onset of renal complications can first be predicted by detection of microalbumin, and the progression of renal disease can

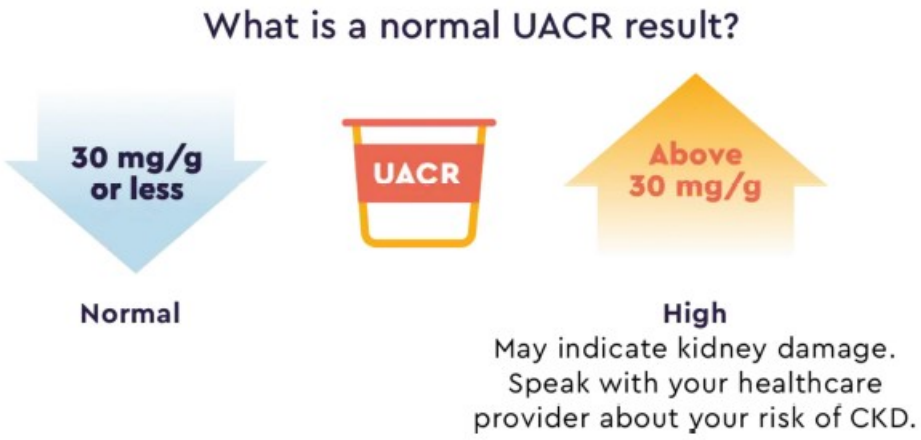
be prevented through better stabilization of blood glucose levels and control of hypertension. The presence of microalbuminuria is also associated with an increased instance of cardiovascular disease.[1]

INDICES:
ACR - Albumin-creatinine ratio.
NAPCR - Non-albumin protein-creatinine ratio.
PCR - Total protein-creatinine ratio.
eGFR - Estimated glomerular filtration rate.
ACR: (albumin-creatinine ratio).

Albumin is a protein in blood. Healthy kidneys generally do not filter it out of the blood, so there should be little to no albumin found in the urine. Albumin/creatinine ratio describes how much albumin is in a urine sample relative to how much creatinine there is. The results are reported as the number of milligrams (mg) of albumin for every gram (g) of creatinine.

- A higher than typical ACR result may be a sign of kidney disease. In particular, the result may indicate a complication of diabetes called diabetic nephropathy or Diabetic Kidney Disease (DKD).

- Glomerular proteinuria results in primary loss of albumin. It is a hallmark of diabetic nephropathy. Measuring albumin in urine is now recommended for the staging of chronic kidney disease (CKD).

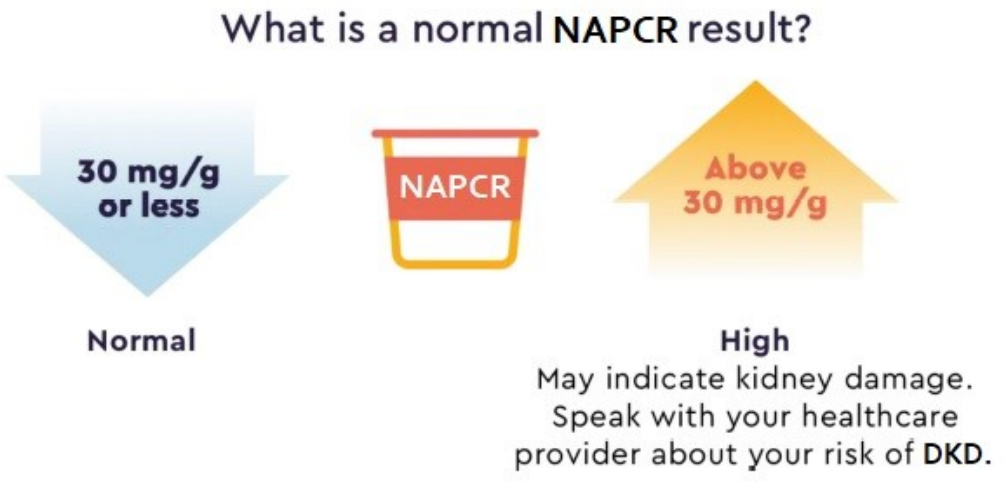


NAPCR: (non-albumin protein-creatinine ratio).

Isolated non-albumin proteinuria (NAP) is a condition in which urine total protein concentrations are elevated without elevation of urine albumin. Glomerular proteinuria is detected by measuring albumin. The other major types of proteinuria, however, may not be detected by the measurement of urine albumin alone. Tubular proteinuria, for

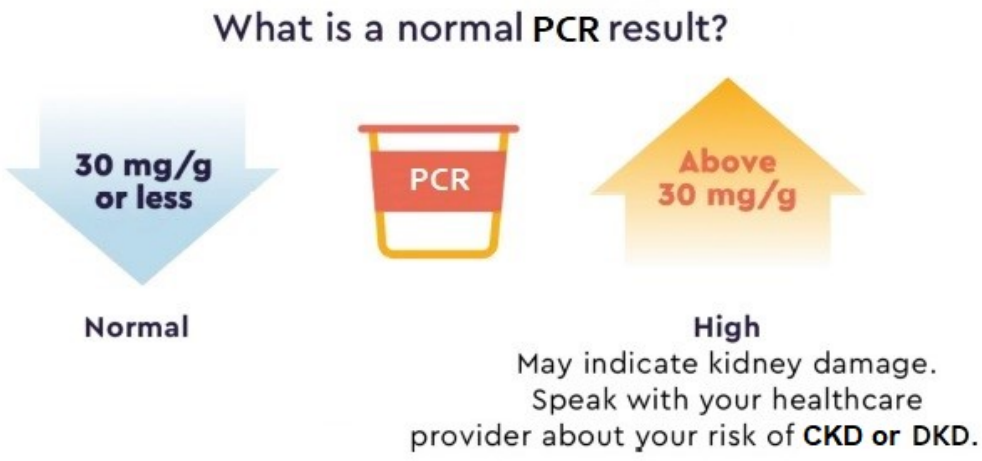
example, can occur from tubulointerstitial disease and decreased reabsorption of low-molecular-weight proteins (e.g., Non-Albumin Proteins, NAP).

Again, the distinct condition when there is no elevation in urine albumin, but the total urine protein concentrations are exceeding the established thresholds is described as isolated non-albumin proteinuria (NAP). NAP is defined (using clinical practice guidelines thresholds) as non-albumin to creatinine ratio (NAPCR) of less than 30 mg/g with a normal ACR level of less than 30 mg/g.



PCR: (total protein-creatinine ratio)

The measurement of (micro) albumin and (micro) non-albumin proteins in urine combined make up the total protein present. The presence of a PCR that is greater than 3 0mg/g is indicative of gross proteinuria.



Important quotes on Gross Proteinuria due to ACR or NAPCR published in Peer Review and Publications.

"Testing for only urine (micro) albumin can miss up to 40% of females and 30.8% of males with gross proteinuria."[2]

"More than 30 million Americans are estimated to be living with CKD, but most aren't aware of their status." - Lee Hilborne, MD, MPH, Past President and Chair of the Appropriate Test Utilization Committee, ASCP.

"The distinct condition when there is no elevation in urine albumin, but the total urine protein concentrations are exceeding the established thresholds is described as isolated non-albumin proteinuria (NAP)."[1]

*"Adds Michael Rocco, MD, MSCE: "We have over half a million people here in the United States who have end-stage kidney disease [ESKD]." The best way to treat it? **"Prevent people from getting ESKD**," says Dr. Rocco, who holds the Vardaman M. Buckalew Jr. chair in internal medicine/nephrology at Wake Forest School of Medicine and is chair of the NKF's Kidney Disease Outcomes Quality Initiative (KDOQI)."*

"Among those considered high risk are patients with hypertension or diabetes mellitus, which includes about 75 million Americans, according to the Centers for Disease Control and Prevention. Multiple data, including from Medicare, the American Medical Group Association, and Optum Clinformatics (a commercial insurance database), suggest that the vast majority—more than 90 percent—of patients with hypertension do not undergo uACR testing; approximately 60 percent of patients with diabetes or with both conditions go untested annually, says Dr. Joseph Vassalotti, chief medical officer of the National Kidney Foundation. Dr. Vassalotti was also quoted as stating that "I've - had so many people tell me how frustrated they are that they didn't know sooner they had kidney disease," he says. "They wish they'd had a chance to do better." (October 2019 CAP TODAY

SO, DON'T NAP ON GROSS PROTEINURIA BY ONLY MEASURING ACR!

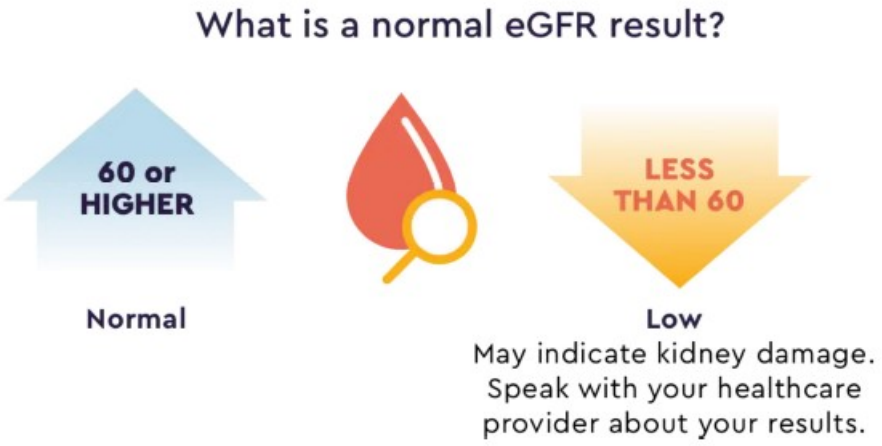
eGFR: estimated glomerular filtration rate

An eGFR test is a blood test that measures how well the kidneys filter waste from the blood and how well the kidneys are functioning. eGFR is a number based on how much creatinine is in the blood.

The measure of serum creatinine may also be used to estimate how quickly the kidneys filter blood (glomerular filtration rate). Because of variability in serum creatinine from

one person to another, the GFR may provide a more accurate reading on kidney function.

The formula for calculating eGFR takes into account the serum creatinine count and other factors, such as age and sex. A GFR score below 60 suggests kidney disease. The range of scores below 60 may be used to monitor treatment and disease progression.



Creatinine (Urine and Serum):

Urine creatinine level

A creatinine urine test measures the amount of creatinine in urine. The test can **help your doctor evaluate how well your kidneys are functioning**. This is useful for diagnosing or ruling out kidney disease and other conditions affecting the kidneys.

Creatinine is of clinical importance because of **its very constant renal clearance**. The determination of the creatinine clearance is a valuable parameter as a measure of the glomerular filtration of the kidney because creatinine is not excreted or reabsorbed by the tubules.

Serum creatinine level

Creatinine usually enters bloodstream and is filtered from the bloodstream at a generally constant rate. The amount of creatinine in blood should be relatively stable. An increased level of creatinine may be a sign of poor kidney function.
Serum creatinine is reported as milligrams of creatinine to a deciliter of blood (mg/dL) or micromoles of creatinine to a liter of blood (micromoles/L). The typical range for serum creatinine is:

- For adult men, 0.74 to 1.35 mg/dL (65.4 to 119.3 micromoles/L)
- For adult women, 0.59 to 1.04 mg/dL (52.2 to 91.9 micromoles/L)

Normalization:

Normalization of Random Urines Submitted for Urinalysis

The use of diuretics and anti-diuretics is common and can potentially lead to dehydration and/or excessive fluid intake which can greatly affect the true urinary analyte concentration. Normalization of urinary analytes corrects for variations in urine concentration.

"Urinary biomarkers, such as albumin and other markers of kidney injury, are frequently reported as a normalized ratio to urinary creatinine (UCr) concentration [UCr] to control for variations in urine flow rate. The implicit assumption is that UCr excretion is constant across and within individuals, such that changes in the ratio will reflect changes in biomarker excretion."[1]

Reporting of Both Quantitative and Normalized Urine Values:
Studies have shown that in cases of non-steady state kidney conditions, such as in individuals who are in onset of Acute Kidney Injury (AKI), in full-blown AKI, or following transplantation, there will typically be changes in urine creatinine excretion rate that may affect results. For this reason, it is best to have both the absolute and normalized values presented to the clinician.

Also, as published in the peer-reviewed Journal of the American Society of Nephrology, "In summary, normalization to urinary creatinine concentration improves the prediction of incipient AKI and outcome but provides no advantage in diagnosing established AKI."[2]

"It is advisable that studies report both absolute and normalized values."[3]

Sciteck reports both the normalized and non-normalized concentrations. This gives the clinician the ability to fully review the urinary constituents and their true normalized and non-normalized absolute urine concentrations to apply on a case-by-case basis.

Normalized Urine Analyte Concentration = $\frac{\text{Analyte Conc. X Creatinine Normal}}{\text{Creatinine Concentration of Sample}}$

Example of Urine Normalization: A random urine with a high creatinine concentration of 375 mg/dL is considered high as compared to the normal population range of 118.8 mg/dL creatinine for women aged 30-39 (see table below). If a woman's random urine has a glucose dipstick value of 2++ to 3+++, this is considered elevated. “The presence of glucose in urine is not a normal finding.”[8] If, however, the urine glucose value is normalized to creatinine, the value could in fact be significantly lower.

Concentrated Urine:
250 mg/dL glucose x 118.8 mg/dL of creatinine / 375 mg/dL creatinine = 79.2 mg/dL glucose – a negative by dipstick. The Average LOD range for Multistix and Chemstrip test strips for glucose is 85.5 mg/dL.[8] The AutoUA® urine glucose LOD is < 1 mg/dL.

Dilute Urine:
250 mg/dL glucose x 118.8 mg/dL creatinine / 20 mg/dL creatinine = 1,485 mg/dL glucose – 4++++ by dipstick. This is considered elevated and may need further investigation.

Normalization as Referenced in Peer-Reviewed Publications:

"Sample normalization should be performed in quantitative metabolomics where the analyzed samples have significant variations in total sample amounts. We believe that sample normalization should be part of the overall metabolomic profiling workflow for quantitative metabolomics, if the sample amount variation is greater than the analytical variation (e.g., ≥±20%)."[4]

"Correcting for urine creatinine level may augment the effectiveness of deterrent programs by mitigating volitional in vivo dilution as a means of subverting workplace drug testing."[5]

"Results confirm the gender-dependence of creatinine concentrations in spot specimens and also show age-dependence, indicating the need for these aspects to be considered when the range of acceptable samples is to be set."[6]

"Finally, because creatinine normalization not only compensates for variable diuresis but also correlates better with body weight-normalized dose of the parent compound, it should be used in biological monitoring of exposure to (PAHs) pyrene and to other substances whose urinary biomarker excretion kinetics parallel that of creatinine."[7]

References:

1.Waikar S., Sabbisetti V., and Bonventre J. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate Kidney Int. 2010 Sep;78(5):486-94.
2. A. Ralib, Pickering, J, Shaw G, Devarjan P. Edelstein C., Bonventre J., and Endre Z. Test characteristics of urinary biomarkers depend on quantitation method in acute kidney injury J Am Soc Nephrol. 2012 Feb;23(2):322-33.
3. Tang, K. W. A.; Toh, Q. C.; Teo, B. W. Normalisation of Urinary Biomarkers to Creatinine for Clinical Practice and Research - When and Why. *Singapore Medical Journal* **2015**, *56* (1), 7–10.
4. Wu Y., Li S., Sample Normalization Methods in Quantitative Metabolomics. J. Chromatography. 2015 Sep:1016.
5. Price J., DO, MPH. Creatinine Normalization of Workplace Urine Drug Tests: Does It Make a Difference? J Addict Med. Vol 7, Number 2, March/April 2013.
6. Carrieri M., Trevisan A., and Bartolucci G.B. Adjustment to concentration-dilution of spot urine samples: correlation between specific gravity and creatinine. Int Arch Occup Environ Health. 2001 Jan;74(1):63-7.
7. Viau C., Lafontaine M., and Payan J.P. Creatinine normalization in biological monitoring revisited: the case of 1-hydroxypyrene. Int Arch Occup Environ Health (2004) 77: 177–185.
8. Ringsrud, K.M., Linne, J.J. Urinalysis and Body Fluids A Color Text and Atlas. Mosby: 1st Ed. 1993.

AutoUA® Creatinine Normalization Table			
Years	All	Male	Female
0-0.5	16	16	16
0.6-1	17.75	17.75	17.75
1-2	60.5	60.5	60.5
3-5	70.75	70.75	70.75
6-11	102.1	104.4	99.48
12-19	161.5	163.6	159.3
20-29	161.8	183	141
30-39	138	157.9	118.8
40-49	124.6	149.7	100.6
50-59	108.1	131.8	86.06
60-69	105.5	126.4	87.91
>70	97.99	117.5	84.51
Average	124.95	141.79	109.71

Barr, D. B.; Wilder, L. C.; Caudill, S. P.; Gonzalez, A. J.; Needham, L. L.; Pirkle, J. L. Urinary Creatinine Concentrations in the U.S. Population: Implications for Urinary Biological Monitoring Measurements. *Environmental Health Perspectives* **2005**, *113* (2), 192–200.

Glucose

The monitoring and detection of diabetes mellitus is important. Under normal circumstances, almost all glucose filtered by the glomerulus is actively re-absorbed in the proximal convoluted tubule, and, therefore, urine contains only minute amounts of glucose.[1] It is well-known that glucose is not normally found in urine.

Tubular re-absorption of glucose is by active transport in response to the body's need to maintain an adequate concentration of glucose. Should the blood level of glucose become elevated (hyperglycemia), as occurs in diabetes mellitus, the tubular transport of glucose has reached it renal threshold and glucose appears in the urine.

Ketones

The term "ketones" represents three intermediate products of fat metabolism, namely, acetone (2%), acetoacetic acid, (20%), and B-hydroxybutyrate (78%).[1] The dipstick only detects acetoacetic acid and does not detect the majority of ketones excreted in urine.

Normally, measurable amounts of ketones do not appear in the urine, because all the metabolized fat is completely broken down into carbon dioxide and water. When, however, the use of available carbohydrate as the major source of energy becomes compromised, body stores of fat must be metabolized to supply energy. Ketones are then detected in urine.[1]

Testing for urinary ketones in most valuable in the management and monitoring of insulin-dependent (type 1) diabetes mellitus. Ketonuria shows a deficiency in insulin, indicating the need to regulate dosage. It is often an early indicator of insufficient insulin dosage in type 1 diabetes and in patients with diabetes who experience medical problems in addition to diabetes.

Increased accumulation of ketones in the blood leads to electrolyte imbalance, dehydration, and if not corrected, acidosis and eventual diabetic coma.

Ketone bodies are produced by the liver and used peripherally as an energy source when glucose is not readily available. The two main ketone bodies are acetoacetate (AcAc) and 3-β-hydroxybutyrate (3HB), while acetone is the third, and least abundant, ketone body. Ketones are always present in the blood and their levels increase during fasting and prolonged exercise. They are also found in the blood of neonates and pregnant women. Diabetes is the most common pathological cause of elevated blood ketones. In diabetic ketoacidosis (DKA), high levels of ketones are produced in response to low insulin levels and high levels of counter regulatory hormones. In acute DKA, the ketone body ratio (3HB:AcAc) rises from normal (1:1) to as high as 10:1. In response to insulin therapy, 3HB levels commonly decrease long before AcAc levels. The frequently employed nitroprusside test only detects AcAc in blood and urine. This test is inconvenient, does not assess the best indicator of ketone body levels (3HB), provides only a semi-quantitative assessment of ketone levels and is associated with false-positive results. Recently, inexpensive quantitative tests of 3HB levels have become available for use with small blood samples (5–25 µl). These tests offer new options for monitoring and treating diabetes and other states characterized by the abnormal metabolism of ketone bodies.

The ketone body ratio, defined as the ratio of circulating 3HB to AcAc, is approximately 1 following a meal, but this rises to nearly 6 after prolonged fasting. The ketone body ratio can also be markedly elevated in diabetic ketoacidosis, alcoholic ketoacidosis, severe hypoxia, end-stage liver disease, hepatic ischemia, various metabolic disorders, and multiple organ failure. All of these pathological states are characterized by changes in the redox potential within hepatocellular mitochondria such that there are low levels of the reduced form of nicotinamide adenine dinucleotide (NADH) and high levels of the oxidized form of nicotinamide adenine dinucleotide (NAD^+).

In addition to the generation of abnormally high levels of ketone bodies in the blood, DKA is also associated with an alteration in the ratio of these two ketone bodies. This ratio rises to 3:1 or higher (to as high as 10:1) in DKA, with relatively high levels of 3HB being generated as a result of the highly reduced state of hepatic mitochondria in the patient with DKA.

Ketone test results can actually cause a false impression that DKA is failing to respond to therapy, when in fact an adequate response is underway. The nitroprusside reagent only detects AcAc, not 3HB. The ketone body ratio in the setting of DKA is initially 3:1 (3HB:AcAc), or greater. As DKA improves with insulin therapy, there is an overall reduction in the levels of ketone bodies and a coincident conversion of 3HB to AcAc, which is driven by an increasingly oxidized state in the hepatocytes. The net effect of these two changes is that AcAc levels tend to plateau for a period of time even as 3HB levels and overall ketone body levels are dropping precipitously. In such a circumstance, the nitroprusside test, whether it is performed on urine or blood, fails to detect the overall improvement, and may lead to unnecessary and potentially dangerous increases in insulin therapy.

Furthermore, ketone tests based on the nitroprusside reaction have been reported to give false-positive results in the presence of drugs containing sulfhydryl groups such as the antihypertensive drug Captopril®, mesna, N-acetylcysteine, dimercaprol, and penicillamine. An overwhelming majority of laboratories fail to follow procedures to eliminate or recognize these false-positive results. Cases in which patients received or nearly received inappropriate therapy with insulin due to false-positive ketone recordings have been reported.

False-negative readings also have been reported when nitroprusside test strips or tablets have been exposed to air for an extended period of time or when urine specimens are highly acidic, such as after the ingestion of large quantities of ascorbic acid. Potentially preventable DKA has been reported in children with Type 1 diabetes due to falsely negative home urine ketone test results.

BHA is a predominant ketone body at the onset of DKA - This test is inconvenient, does not assess the best indicator of ketone body levels (3HB) The frequently employed nitroprusside **test** only detects AcAc in blood and urine. This **test is inconvenient**, **does not assess the best indicator of ketone body levels**(**3HB**), provides only a semi quantitative **assessment** of **ketone levels** and is associated with false-positive results. - Dec 8, 2017 ketone body (CHEBI:73693) - EMBL-EBI

Elevated plasma beta-hydroxybutyrate concentrations without ketonuria in healthy insulin-dependent diabetic patients published by MacGillivray MH, Li PK, Lee JT, Mills BJ, Voorhess ML, Putnam TI, Schaefer PA., in the Journal of Endocrinology stated the following abstract.

Abstract

Plasma beta-hydroxybutyrate (beta-OHB) concentrations and simultaneous urine tests for ketonuria (nitroprusside reaction) were evaluated every 4 h throughout a 24-h study in 10 healthy insulin-dependent diabetics who had poor control based on home urine tests and elevated hemoglobin A1C. Concurrent measurements of the major carbohydrate regulatory hormones were made in the diabetic group and in a control population of 20 age-matched subjects. In the diabetics, 73% of the beta-OHB measurements were elevated. Only 43% of the abnormal beta-OHB values were associated with ketonuria. The diabetic subjects also showed exaggerated diurnal patterns for plasma beta-OHB and cortisol. There were no significant differences for the other regulatory hormones in the diabetic and normal groups. We conclude that 1) abnormal plasma beta-OHB levels without ketonuria are prevalent in poorly controlled diabetics; 2) **negative nitroprusside tests for ketonuria underestimate the presence of ketonemia due to increased beta-OHB concentrations**; 3) both insulin deficiency and glucocorticoid excess may influence ketone body metabolism in insulin-dependent diabetic patients. - J Clin Endocrinol Metab. 1982 Mar;54(3):665-8.

The concentrations in urine for *B*-hydroxybutyrate and acetoacetic acid in blood and urine are as follows with the corresponding research and reference ranges below. There are dozens more articles to support the ranges and the importance of this testing.

As a note, I would like to add in our research on this subject that multiple articles have stated that the presence of *B*-hydroxybutyrate (BOHB) in urine may cause **kidney damage**. These studies were mainly focused on inducing ketosis for the study of ketone excretion, and they wanted to warn future researchers of the potential for kidney damage. So, the presence of BOHB is significant for many reasons.

In the alternative, according to ***Ketones: Metabolism's Ugly Duckling*****,** Authored by T. VanItallie and T Nufert, B.A. in *Nutrition Reviews, Vol. 61, No. 10* the following was stated:

"Diet-induced hyperketonemia (at any level) will prove clinically beneficial in the management of a number of neurodegenerative disorders, particularly Parkinson's disease and Alzheimer's disease."

Ketone Ranges:
Urine *B*-hydroxybutyrate: 0 - 25 mg/dL (0 - 1.4 mmol/L)
Note: Levels of >50 mg/dL further evaluation may be needed immediately.
Urine Acetoacetate: 0 to 5 mg/dL (0 - 0.3 mmol/L)

Blood *B*-hydroxybutyrate: 7.2 - 9.0 mg/dL (0.4 - 0.5mmol/L)
Note: DKA levels are usually more than 3 mmol/L

Important Notes and Comments in Peer Review Research and Publications:

"*In our study, there were four patients who were diagnosed with DKA according to the ADA criteria. In spite of the fact that significant ketonemia was determined in these patients, the urine dipsticks only identified significant ketonuria ("3+") in one of these patients."* - Kuru, B.; Sever, M.; Aksay, E.; Dogan, T.; Yalcin, N.; Eren, E. S.; Ustuner, F. Comparing Finger-Stick B-Hydroxybutyrate with Dipstick Urine Tests in the Detection of Ketone Bodies. *Turkish Journal of Emergency Medicin* **2014**, *14* (2), 47–52.

"*In contrast to these striking advances in the approach to glucose monitoring, the process by which ketone bodies are measured in urine and blood, and the clinical indications for doing so have not changed significantly in 25 years*." "Can the direct measurement of 3HB enhance the management of DKA in any way? B-Hydroxybutyrate levels correlate better than AcAc with changes in acid-base status during the course of treatment for DKA." - Laffel, L. Ketone Bodies: a Review of Physiology, Pathophysiology and Application of Monitoring to Diabetes. *Diabetes/Metabolism Research and Reviews* **1999**, *15*, 412–426.

"*The major pitfall for testing ketone bodies with stick test is that the test detects only Ac and AcAc and, not BHB. In ketoacidosis, BHB is the main ketone body as the ratio between BHB and AcAc, being normally ~1:1, shifts strongly toward BHB*." - Urbain, P.; Bertz, H. Monitoring for Compliance with a Ketogenic Diet: What Is the Best Time of Day to Test for Urinary Ketosis? *Nutrition & Metabolism* **2016**, *13* (77).

"*For the optimal clinical management of children with epilepsy on a KD, the International Ketogenic Diet Study Group recommends monitoring compliance by urine testing for ketosis several times a week.*" - Walta, A.-M.; Keltanen, T.; Lindroos, K.; Sajantila, A. The Usefulness of Point-of-Care (POC) Tests in Screening Elevated Glucose and Ketone Body Levels Postmortem. *Forensic Science International* **2016**, *266*, 299–303.

"*The urinary excretion of βHB abruptly increased in group 3 (more than 1.8mM). It was 100 times higher than the blood βHB concentration." "Further studies are necessary to establish the safety of fasting as a way to induce hyperketosis for the therapeutic needs. Our observations of a slight decrease in hepatic function and a 12% decrease in eGFR call for action*." - Watanabe, S.; Hirakawa, A.; Utada, I.; Aoe, S.; Moriyama, S.; Honda, H.; Takiguchi, R.; Haba, R.; Hoshi, T. Ketone Body Production and Excretion During Wellness Fasting. *Diabetes Research* **2017**, *3* (1), 1–8.

References:

1. Historical Reference: Siemens Multistix® (insert attached) - Reference range for lowest detectable Acetoacetate by dipstick is 5 to 10 mg/dL (0.3 mmol/L to 0.6 mmol/L). Research from multiple Peer review research articles, Reference Manuals on Urinalysis and other sources have confirmed that Ketones present in urine are in the following concentrations if present: 78% B-hydroxybutyric acid (BOHB), 20% Acetoacetic acid (Ac) and 2% Acetone. Essentially the references insert for urinalysis from Siemens, Miles and other manufactures have a claimed low end of detection for the presence of Ketones in the urine. Please note that dipsticks will only pick up the minor portion of Ketone Bodies in the urine in the form of Acetoacetic acid.
2. Determination of B-Hydroxybutyrate in Blood and Urine by GCMS; Journal of Analytical Tox, Vol 13, 2009. For BOHB measured in urine of the post-mortem cases 7 out of the 12 that were measured for BOHB had levels of BOHB that were greater than >500 mg/dL (>50 mg/dL).
3. Urinary Excretion of Beta-Hydroxybutyrate and Acetoacetate During Experimental Ketosis; Journal of Experimental Physiology, Vol 53, 181-193, 1968. States that "The two ketone bodies (e.g., BOHB and Ac) had a constant ration in plasma, but, as ketosis progresses, beta-hydroxybutyrate because the preponderant molecule in the urine." And stated that "as the ketosis increases in severity, BHOB becomes increasingly the predominant molecule in the urine, while the ratio of BOHB to AC in the plasma remains virtually constant."
4. Beside Detection of Urine B-Hydroxybutyrate in Diagnosing Metabolic Acidosis; Society for Academic Emergency Medicine, Pubmed/18637083, 2008. Given that elevated BOHB may be a marker for sudden death in AKA may indicate severe DKA and account for up to 70% of the total Ketones in other toxicologic ketoses". The paper goes on to state that "In other words, at 40 mmol/L BOHB (the limit of consistent detection)" - by dipstick - "even a severe 10:1 BOHB:AcAc ration would imply that 4.0 mmol AcAc is already present." This implies that using a dipstick to try to measure BOHB by the addition of H202 and to ingredients that the lowest sensitivity is significantly above values already indicative of Ketosis.

5. New automated and high-throughput quantitative analysis of urinary ketones by multifiber exchange-solid phase microextraction coupled to fast gas chromatography/negative chemical-electron ionization/mass spectrometry; Journal Automated Methods Manag Chemistryl Pubmed/20628512, 2010. As stated in the paper "To demonstrate the applicability of the methods to urinary samples, the content of these compounds in human urine (n = 20) of no-exposed, no-smoking subject was analyzed. The urinary excretion of Ace (acetone), BOHB, and AcAc were excreted at a high concentration of (0.11 - 0.38 mg/L)." - essentially zero (0 mg/dL). "Concentrations of HOHB 183-2300 mg/L, AcAc (40 - 872 mg/L), and Ac (10 - 431 mg/L) in urine test stick positive (score + - ++++) were obtained." That is to say the range for dipsticks from 1 + top 4 + ranges anywhere from 0 for normals (non-smoking,) to 230 mg/dL for a 4+ dipstick result which only shows the Ac (acetoacetic acid) value or 87 mg/dL. The ratio of BOHB of 78%, 20% Ac and 2% Acetone if further supported as well as the insensitivity of the dipsticks to the presence of ketones.
6. Historical Reference Range Siemens Mulstix® Insert: Sensitivity: 5-10 mg/dL
Performance Characteristics: The test reacts with acetoacetic acid in urine. It does not react with acetone or *B*-hydroxybutyric acid.
7. Lose Weight by Achieving Optimal Ketosis; Ketone Measuring Weight Loss, Dr. Andreas Eienfeldt, MD, 03/13/13.
Dr. Eigfeldt states that "Below 0.5 mmol/L is not considered "ketosis." - below 9 mg/dL acetoacetic acid.
8. Alcoholic Ketoacidosis; Schwiz Med Wochenschr. 1993 Oct 16;123(41): 1929-34. States that "Routine testing for urine ……..with ketostix may be negative since they do not detect beta-hydroxybutyrate and this is characteristically elevated in AKA.
9. Urinary Ketone Bodies and their Ratios; Urinalysis & Body Fluid, Strasinger 3rd Ed F.A. Davidson Pub, Mosbly, 2004. Urinary Ketone Bodies and their ratios are as follows: "78% B-hydroxybutyric acid (BOHB), 20% Acetoacetic acid (Ac) and 2% Acetone".
10. Urinary Ketone Bodies and their Ratios; Urinalysis & Body Fluid, Strasinger 6th Ed F.A. Davidson Pub, Mosbly, 2014. Urinary Ketone Bodies and their ratios are as follows: "78% B-hydroxybutyric acid (BOHB), 20% Acetoacetic acid (Ac) and 2% Acetone".
11. Diabetic Ketoacidosis - American Family Physician; "Beta-hydroxybutyrate accounts for about 75% of ketones in ketoacidosis" - in blood.
12. Ketone Body Production and Excretion During Wellness Fasting; Diabetes Research, Lifescience Promoting Ass, ISSN 2379-6375, 2017.
Ranges during this study:
Urine BOHB (Normal): 0 - 15.1 mM
Urine BOHB (Elevated): 2.72 - 687.0 mM
Notes during study: "BOHB excreted in urine may cause renal damage." This was the second study to directly state that the excretion of B-hydroxybutyrate may damage kidneys.

Blood (hemoglobin - rbcs)

Blood may be present in the urine in either in the form of intact red blood cells (hematuria) or as the product of red blood cell destruction (lysis), hemoglobin (hemoglobinuria). The finding of a positive reagent strip test or a test for hemoglobin in urine indicates the presence of red blood cells (rbcs), hemoglobin or myoglobin. Each of these finding has a different clinical significance.

Hematuria. Hematuria is most closely related to disorders of renal or genitourinary origin in which bleeding is the result of trauma or damage to the organs of these systems. Major causes of hematuria included renal calculi, glomerular diseases, tumors, trauma, pyelonephritis, exposure to toxic chemicals and anti-coagulant therapy.

Hemoglobinuria. Hemoglobinuria may result from the lysis of red blood cells produced in the urinary tract. It may also be the result of intravascular hemolysis and the subsequent filtering of hemoglobin through the glomerulus.

Myoglobinuria. Myoglobinuria, a heme-containing protein found in muscle tissue. The presence of myoglobin rather than hemoglobin should be suspected in patients with conditions associated with muscle destruction (rhabdomyolysis).

Bilirubin

The presence of bilirubin in urine can provide an early indication of liver disease. It is often detected long before the donor exhibits jaundice.

Bilirubinuria. Only conjugated bilirubin can appear in the urine when the normal degradation cycle is disrupted by bile duct obstruction (post-hepatic jaundice) as a result of for example gallstones or cancer or when the integrity of the liver is damages (hepatic jaundice). Hepatitis and cirrhosis are common example of conditions that produce liver damage.

Urobilinogen

When conjugated bilirubin is excreted through the bile duct into the intestine, the intestinal bacteria convert the bilirubin to a combination of urobilinogen and stercobilinogen. Some of the urobilinogen is reabsorbed form the intestine into the blood, recirculated to the liver, and is excreted back into the intestine through the bile duct.

Increase in urobilinogen (greater than 1mg/dL) is seen in liver disease and hemolytic disorders. The detection of urobilinogen is important in the early detection of liver disease. Elevation could be caused by constipation.

Absence of urobilinogen in the urine and feces is also diagnostics significant and represents an obstruction of the bile duct that prevents the normal passage of bilirubin into the intestine. **The absence of urobilinogen cannot be detected by a dipstick**.[1] The AutoUA® quantitative urobilinogen can, however, detect the absence of urobilinogen.

"The absence of urobilinogen indicating bile duct obstruction cannot be detected by a dipstick."

Nitrite

The AutoUA® reagent assay for nitrite provides a rapid screening test for the presence of urinary tract infection (UTI). The nitrite assay is not intended to replace the urine as the primary test for diagnosis and monitoring bacterial infection. Many UTIs are believed to start in the bladder as a result external contamination and, if untreated, progress upward through the ureters to the tubules, renal pelvis, and kidney. The nitrite if valuable for detecting initial bladder infection (cystitis) because patients are often asymptomatic or have vague symptoms that would not lead the physician to order a urine culture.

The nitrite assay detects gram-negative bacteria (containing reductase) that most frequently cause UTIs. Reductase is the enzyme responsible for reducing nitrate to nitrite. Non-nitrate gram positive bacteria and yeasts, however, cause a significant number of infections.

Specific Gravity

The ability of the kidneys to concentrate glomerular filtrate by selectively reabsorbing essential chemicals and water from the glomerular filtrate is one of the kidneys most important functions, hence the importance of evaluating urine concentration. Normal ranges for specific gravity in human urine is from approximately 1.005 to 1.035 specific gravity units. The specific gravity of human urine can vary widely based on intake of fluid and or dehydration. Creatinine is, therefore, a better marker for true urine concentration as it related to normalization of the urine value.

Leukocyte Esterase

Leukocyturia. Prior to the development of the AutoUA® quantitative Leukocyte Esterase (wbc) assay detection assay, the subjective and variable methods of dipstick and microscopic detection were relied upon. This can be subject to variation from method used, read times, technical skill, etc. The true automation of this method offers a true method to standardize the detection of leukocytes.

"*The use of a method for the detection of leukocyte esterase and nitrite to determine the necessity of performing urine cultures can be a cost effective measure*." - Urine leukocytes and nitrite tests as an aid to predict urine culture results. Lab Med 15(3):186-187, 1984.

From CodeMap® - only the use of the leukocyte and nitrite test kit for bacteriuria screen can be used for reimbursement for CPT code: 81007-59* Urinalysis; bacteriuria screen except by culture or dipstick. The use of dipstick and/or urine cultures are not allowed for use with this code.

CodeMap[1] also suggests that the Creatinine CPT code is: 82570. Creatinine is required for all AutoUA® normalization including for nitrite and leucocyte esterase for increased accuracy and specificity for bacteremia screening.
*A -59 modifier is used to indicate that this is a separate test and not a duplicate since the same CPT code is used to report nitrite.

UTI:

The term “pyuria” literally means “pus in the urine” but, in common usage, the focus is not on the presence of pus but rather on the number of white blood cells (WBCs) or on an amount of leukocyte esterase (LE) that exceeds a threshold and suggests a urinary tract infection (UTI). In the journal of Pediatrics, Chaudhari et al. state the following, “Urine concentration should be incorporated into the interpretation of automated microscopic urinalysis in young infants.”[2] The results of this study on the impact of urine concentration provide the optimal threshold for a new era of automated urinalysis. As referenced by Chaudhari et al.,[2] urinalysis provides a practical time window for clinicians to render prompt treatment. Dr. Kenneth B. Roberts, MD also references Chaudhari et al.[2] and states that the findings “provide valuable assistance for interpreting the results of automated urinalyses.”[3] Roberts[3] goes on to suggest that using urinalysis as a screen for UTI permits selecting individuals for antimicrobial treatment 24 hours sooner than if clinicians were to wait for culture results prior to treatment.

References:

1. CodeMap® coding and reimbursement for Sciteck®’ Quantitative Urinalysis Assays – attached.
2. Chaudhari et al. Journal of Pediatrics; 2016 Nov; 138(5).
3. Dr. Kenneth B. Roberts, MD. Journal of Pediatrics; Vol 158, No. 5, November 2018.