KETONE TESTING

1. Use

> **Recommendation:** Ketones should be measured in urine or blood by patients with diabetes in the home setting and in the clinic/hospital setting as an adjunct to the diagnosis of diabetic ketoacidosis.
>
> Level of evidence: E

The ketone bodies, acetoacetate (AcAc), acetone, and β-hydroxybutyric acid (βHBA), are catabolic products of free fatty acids. Determinations of ketones in urine and blood are widely used in the management of patients with diabetes mellitus as adjuncts for both diagnosis and ongoing monitoring of diabetic ketoacidosis (DKA). Measurements of ketone bodies are routinely performed both in an office/hospital setting and by patients at home.

The ADA recommends that initial evaluation of patients with diabetes mellitus include determination of urine ketones and that urine ketone testing should be available in the physician's office for immediate use as needed (14). The ADA further recommends that urine ketone testing is an important part of monitoring by patients with diabetes, particularly in those with type 1 diabetes, pregnancy with preexisting diabetes, and GDM (9). All patients with diabetes mellitus should test their urine for ketones during acute illness, stress, persistent hyperglycemia [plasma glucose >16.7 mmol/L (300 mg/dL] pregnancy, or symptoms consistent with DKA, such as nausea, vomiting, or abdominal pain (9, 14).

2. Rationale

Ketone bodies are normally present in urine and blood, but in very low concentrations (e.g., total serum ketones <0.5 mmol/L). Increased ketone concentrations in patients with known diabetes mellitus or in previously undiagnosed patients presenting with hyperglycemia suggest impending or established DKA, a medical emergency. The two major mechanisms for the high ketone concentrations in patients with diabetes are increased production from triglycerides and decreased utilization in the liver, both a result of absolute or relative insulin deficiency and increased counter-regulatory hormones including cortisol, epinephrine, glucagon, and growth hormone (118).

The principal ketone bodies βHBA and AcAc are normally present in approximately equimolar amounts. Acetone, usually present in only small quantities, is derived from spontaneous decarboxylation of AcAc. The equilibrium between AcAc and βHBA is shifted towards formation of βHBA in any condition that alters the redox state of hepatic mitochondria to increase concentrations of NADH such as hypoxia, fasting, metabolic disorders (including DKA) and alcoholic ketoacidosis (119-121). Thus, assay methods for ketones that do not include measurement of βHBA may provide misleading clinical information by underestimating total ketone body concentration (90, 122).

3. Analytical Considerations

A. Urine ketones

1. Pre-analytical: Normally, the concentrations of ketones in the urine are below the detection limits of commercially available testing materials. False-positive results have been reported with highly colored urine and in the presence of several sulfhydyl containing drugs, including angiotensin-converting enzyme inhibitors (123). Urine test reagents deteriorate with exposure to air, giving false-negative readings; testing material should be stored in tightly sealed containers and discarded after the expiration date

on the manufacturer's label (124). False-negative readings have also been reported with highly acidic urine specimens, such as after large intakes of ascorbic acid. Loss of ketones from urine attributable to microbial action can also cause false-negative readings. Since acetone is a highly volatile substance, specimens should be kept in a closed container. For point-of-care analyses in medical facilities and for patients in the home setting, control materials (giving both negative and positive readings) are not commercially available but would be desirable to assure accuracy of test results.

2. Analytical: Several assay principles have been described. Most commonly used is the colorimetric reaction that occurs between ketones and nitroprusside (sodium nitroferricyanide), resulting in a purple color (26). This method is widely available in the form of dipsticks and tablets and is used to measure ketones in both urine and blood (either serum or plasma). Several manufacturers offer dipsticks that measure glucose and ketones; a combination dipstick is necessary only if the patient monitors urine glucose instead of or in addition to blood glucose. The nitroprusside method measures only AcAc unless the reagent contains glycine, in which case acetone is also measured. The nitroprusside-containing reagent is much more sensitive to AcAc than acetone with respect to color generation. Importantly, this reagent does not measure βHBA (122).

B. Blood ketones

1. Preanalytical: Serum/plasma ketones can be measured using tablets or dipsticks routinely used for urine ketone determinations. Although specimens can be diluted with saline to "titer" the ketone level (results are typically reported as "positive at a 1/x dilution"), as with urine ketone testing, βHBA, the predominant ketone body in DKA, is not detected.

   For specific determinations of βHBA, as described below, specimen requirements differ among methods. In general, blood samples can be collected into heparin, EDTA, fluoride, citrate or oxalate (for the BioScanner Ketone system, fluoride and oxalate have not been tested according to the manufacturer). Ascorbic acid interferes with some assay methods. AcAc interferes with some assay methods unless specimens are highly dilute. Specimen stability differs among methods, but in general, whole blood specimens are stable at 4˚C for up to 24 h. Serum/plasma specimens are stable for up to one week at 4˚C and for at least several weeks at –20˚C (long-term stability data are not available for most assay methods).

2. Analytical: Although several different assay methods (e.g., colorimetric, gas chromatography, capillary electrophoresis and enzymatic) have been described for blood ketones, including specific measurement of βHBA, enzymatic methods for quantification of βHBA appear to be the most widely used for routine clinical management (125-127). The principle of the enzymatic methods is that βHBA in the presence of NAD is converted to AcAc and NADH by ß-hydroxybutyrate dehydrogenase. Under alkaline conditions (pH 8.5-9.5), the reaction favors formation of AcAc from βHBA. The NADH produced can be quantified spectrophotometrically (usually kinetically) with use of a peroxidase reagent (Analox Instruments U.S.A., Lunenburg, MA). One manufacturer offers a method that utilizes a test card impregnated with the reagents (KetoSite, GDS Diagnostics, Elkhart, IN). Most methods permit use of whole blood, plasma, or serum specimens (required volumes are generally 200 mL or less). Some methods permit analysis of multiple analytes and are designed for point-of-care testing. Several methods are available as hand-held meters, which are FDA-approved for both laboratory use and for over-the-counter use by patients (e.g., BioScanner Ketone, PolymerTechnology Systems, Indianapolis, IN; MediSense Precision Xtra, Abbott Laboratories, Abbott Park, IL) (127). These methods utilize dry chemistry test strips to which a drop of whole blood, serum, or plasma is added. Results are displayed on the instruments within approximately 2 minutes.

4. Interpretation

A. Urine ketone determinations

> **Recommendation:** Urine ketone determinations should not be used to diagnose or monitor the course of DKA.
>
> Level of evidence: A

In a patient with known diabetes mellitus or in a patient not previously diagnosed with diabetes but who presents with typical symptoms of diabetes and hyperglycemia, the presence of positive urine ketone readings suggests the possibility of impending or established DKA. Although DKA is most commonly associated with type 1 diabetes mellitus, it may rarely occur in type 2 patients (128). Patients with alcoholic ketoacidosis will have positive urine ketone readings, but hyperglycemia is not usually present. Positive urine ketone readings are found in up to 30% of first morning urine specimens from pregnant women (with or without diabetes), during starvation, and after hypoglycemia (90, 122, 129).

B. Blood ketone determinations

> **Recommendation:** Blood ketone determinations that rely on the nitroprusside reaction should be used only as an adjunct to diagnose DKA and should not be used to monitor treatment of DKA. Specific measurement of βHBA in blood can be used for diagnosis and monitoring of DKA. Further studies are needed to determine if the test offers any clinical advantage over more traditional management approaches (e.g., measurements of serum CO_2, anion gap, or pH).
>
> Level of evidence: E

Blood ketone determinations that rely on the nitroprusside reaction should be used with caution for diagnosis of DKA as results do not quantify βHBA, the predominant ketone in DKA. The test should not be used to monitor the course of therapy since AcAc and acetone may increase as βHBA falls during successful therapy (90, 118-122). Blood ketone determinations that measure βHBA specifically are useful for both diagnosis and ongoing monitoring of DKA (121, 130-132). Reference intervals for βHBA differ among assay methods, but concentrations in healthy individuals fasted overnight are generally <0.5 mmol/L. Patients with well-documented diabetic ketoacidosis [serum CO_2 <17 mmol/L, arterial pH <7.3, plasma glucose >14.9 mmol/L (250 mg/dL)] generally have βHBA concentrations >2 mmol/L.

Further studies are also needed to determine if blood ketone determinations by patients with diabetes mellitus are preferable (e.g., better accepted by patients than urine testing, more prompt diagnosis of DKA) to urine ketone determinations.