Chapter 8

Ketone Testing

1. USE

Recommendation

Ketones measured in urine or blood in the home setting by patients with diabetes and in the clinic/hospital setting should be considered only an adjunct to the diagnosis of DKA. GPP

The ketone bodies acetoacetate (AcAc), acetone, and β-hydroxybutyric acid (βHBA) are catabolic products of free fatty acids. Measurements of ketones in urine and blood are widely used in the management of patients with diabetes as adjuncts for both diagnosis and ongoing monitoring of DKA. Measurements of ketone bodies are routinely performed, both in an office/ hospital setting and by patients at home. The ADA recommends that ketosis-prone patients with diabetes check urine or blood ketones in situations characterized by deterioration in glycemic control in order to detect and preempt the development of DKA (*21, 182*).

2. RATIONALE

Ketone bodies are usually present in urine and blood, but in very low concentrations (e.g., total serum ketones, <0.5 mmol/L). Increased ketone concentrations detected in patients with known diabetes or in previously undiagnosed patients presenting with hyperglycemia suggest impending or established DKA, a medical emergency. The 2 major mechanisms for high ketone concentrations in patients with diabetes are increased production from triglycerides and decreased utilization in the liver— both of which are due to an absolute or relative insulin deficiency and increased counter-regulatory hormones, including cortisol, epinephrine, glucagon, and growth hormone (*183*).

The principal ketone bodies βHBA and AcAc are typically 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 βHBA formation in any condition that alters the redox state of hepatic mitochondria to increase NADH concentrations, such as hypoxia, fasting, metabolic disorders (including DKA), and alcoholic ketoacidosis *(184–186)*. Thus, assay methods for ketones that do not include βHBA measurement may provide misleading clinical information by underestimating total ke-tone body concentration *(187)*.

3. ANALYTICAL CONSIDERATIONS

A. Urine ketone. Preanalytical. The concentrations of ketones in the urine of healthy individuals 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 sulfhydryl-containing drugs, including angiotensin-converting enzyme inhibitors (*188*). Urine test reagents deteriorate with exposure to air, giving false-negative readings; therefore, testing material should be stored in tightly sealed containers and discarded after the expiration date on the manufacturer's label (*189*). False-negative readings have also been reported with highly acidic urine samples, such as after large intakes of ascorbic acid. Loss of ketones from urine attributable to microbial action can also cause false-negative readings. Because acetone is a highly volatile substance, samples should be kept in a closed container. For point-of-care analyses in medical facilities and for patients in the home setting, control materials (that give both negative and positive readings) are not commercially available but would be desirable to ensure accuracy of test results.

Analytical. Several assay principles have been described. Most commonly used is the colorimetric reaction that occurs between AcAc and nitroprusside (sodium nitroferricyanide) to produce a purple color (*181*). This method is widely available in the form of dipsticks and tablets and is used to measure ketones in both the urine and blood (either serum or plasma). Several manufacturers offer dipsticks for measuring 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 cannot be used to measure βHBA (*181*).

B. Blood ketones. Preanalytical. Serum/plasma ketones can be measured with the tablets or dipsticks routinely used for urine ketone measurements. Although samples can be diluted with saline to "titer" the ketone concentration (results are typically reported as "positive at a $1/x$ dilution"), βHBA, the

predominant ketone body in DKA, is not detected, as with urine ketone testing.

For specific βHBA measurements, sample requirements differ among methods, as is described below. In general, blood samples can be collected into tubes containing heparin, EDTA, fluoride, citrate, or oxalate. Ascorbic acid interferes with some assay methods. AcAc interferes with some assay methods unless the samples are highly dilute. Sample stability differs among methods, but whole-blood samples are generally stable at 4 °C for up to 24 h. Serum/plasma samples are stable for up to 1 week at 4 °C and for at least several weeks at −20 °C (long-term stability data are not available for most assay methods).

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 appear to be the most widely used for the quantification of βHBA for routine clinical management *(190–192)*. The principle of the enzymatic methods is that β-hydroxybutyrate dehydrogenase in the presence of NAD+ converts βHBA to AcAc and NADH. Under alkaline conditions (pH 8.5–9.5), the reaction favors the formation of AcAc from βHBA. The NADH produced can be quantified spectrophotometrically (usually kinetically) with the use of a peroxidase reagent. Most methods permit the use of whole blood, plasma, or serum samples (required volumes are generally ≤200 μL). Some methods permit the analysis of multiple analytes; these methods are designed for point-of-care testing. Several methods are available as handheld meters, which have been FDA cleared for both laboratory use and home use by patients. These methods use 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 min.

4. INTERPRETATION

> ***Recommendation***
>
> Urine ketone measurements should not be used to diagnose or monitor the course of DKA.
> GPP

A. Urine ketone measurements. The presence of positive urine ketone readings in a patient with known diabetes or a patient not previously diagnosed with diabetes but who presents with typical symptoms of diabetes and hyperglycemia suggests the possibility of impending or established DKA. Although DKA is most commonly associated with type 1 diabetes, it may occur rarely in type 2 patients *(193)*. Patients with alcoholic ketoacidosis will have positive urine ketone readings, but hyperglycemia is not usually present. Positive urine ke-tone readings are found in up to 30% of first morning urine samples from pregnant women (with or without diabetes), during starvation, and after hypoglycemia *(187)*.

> ***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 DKA treatment. Specific measurement of βHBA in blood can be used for diagnosis and monitoring of DKA.
> B (moderate)

B. Blood ketone measurements. Blood ketone measurements that rely on the nitroprusside reaction should be used with caution for DKA diagnosis, because the results do not quantify βHBA, the predominant ketone in DKA. The test should not be used to monitor the course of therapy, because AcAc and acetone may increase as βHBA decreases during successful therapy *(147, 183–187)*. Blood ketone measurements that measure βHBA specifically are useful for both the diagnosis and ongoing monitoring of DKA *(194–196)*. Reference intervals for βHBA differ among assay methods, but concentrations in healthy individuals who have fasted overnight are generally <0.5 mmol/L. Patients with well-documented DKA [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.

5. EMERGING CONSIDERATIONS

Further studies are needed to determine whether blood ketone measurements by patients with diabetes are preferable (e.g., better accepted by patients, more prompt diagnosis of DKA) to urine ketone measurements. Studies are necessary to evaluate whether the test offers any clinical advantage over more traditional management approaches (e.g., measurements of serum CO_2, anion gap, or pH).