New guidance on lab analysis in diabetes

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December 2023—The third and latest edition of recommendations for laboratory analysis in diagnosing and managing diabetes mellitus, released this summer, provide guidance on, among other things, ketone testing, glycolysis, and point-of-care testing (Sacks DB, et al. *Diabetes Care.* 2023;46[10]:e151–e199; Sacks DB, et al. *Clin Chem.* 2023;69[8]:808–868). The last such recommendations were published in 2011.

David B. Sacks, MB, ChB, senior investigator and chief of clinical chemistry, National Institutes of Health Department of Laboratory Medicine, and a member of the CAP Clinical Chemistry Committee, was chair of the expert committee that compiled the evidence, invited review, presented the recommendations for public comment, and obtained approval from the American Association for Clinical Chemistry (now ADLM) and American Diabetes Association. He was first asked nearly 25 years ago to chair a committee of experts to establish laboratory analysis guidelines. "I said, 'There's no point in doing this unless the American Diabetes Association participates,'" he tells CAP TODAY.

Dr. Sacks convinced the ADA of its importance, and it has been an active participant since the first laboratory analysis guidelines were published in 2002. The ADA is "very strongly behind this," he says. The more than 80 recommendations in the latest publication have the ADA's endorsement. "They carry a lot of weight," he says.

All of the authors of the 2011 guidelines returned to work on the update. The group consists of five patient-facing physicians, one chemist, and three clinical laboratory medicine experts—Dr. Sacks, David Bruns, MD, of the University of Virginia School of Medicine, and Andrea R. Horvath, MD, PhD, of Prince of Wales Hospital in Sydney.

The recommendations are graded for their strength and rated for the quality of the underlying body of evidence. "Many don't have quite as strong a recommendation because the studies that were evaluated were not designed to look at the performance of the lab test," Dr. Sacks says. "Most of the studies are clinical."

Some recommendations advise against tests that lack evidence for their use—for example, routine use of blood glucose meters for people with type 2 diabetes treated with diet and/or oral agents alone. "There's no evidence to support it," Dr. Sacks says. The evidence at the time of the 2011 guidelines "was debatable, and some people said it was useful," he says. But studies published since then have shown "it isn't, so therefore the recommendation is, 'Don't do it.'"

On the topic of ketone testing, the recommendation for diagnosis of diabetic ketoacidosis reinforces earlier recommendations in advising the specific measurement of beta-hydroxybutyrate (βOHB) in blood. The measurement may also be used for monitoring during treatment of DKA.

"People should measure only beta-hydroxybutyrate and not total ketones because that's a much better reflection of the state of ketosis in the blood," Dr. Sacks explains.

Dr. Sacks and coauthors last year detailed the controversies around the measurement of blood ketones to diagnose and manage diabetic ketoacidosis (Kilpatrick ES, et al. *Diabetes Care.* 2022;45[2]:267–272). In their article they reported that CAP records revealed "a split in what is measured, such that, in 2020 (KET-04), 1,785 laboratories were measuring specifically BOHB while 840 were measuring a reaction using nitroprusside. In contrast," they wrote, "the equivalent quality schemes in the U.K. show that no laboratories measure blood ketones using the nitroprusside test."

Dr. Sacks' European coauthors of that article and others "were shocked that in the U.S.," he says, "so many labs use total ketones and don't measure beta-hydroxybutyrate."

In their 2022 article on the controversies, they wrote that the nitroprusside test principally measures acetoacetate,

not βOHB, and since βOHB predominates in ketoacidosis, the degree of ketonemia using nitroprusside initially could be underestimated. In addition, they said, when the acidosis has resolved, βOHB is more readily oxidized to acetoacetate, "so overall ketosis paradoxically may appear to be worsening when the converse is true."

Blood ketone βOHB can be measured not only in the laboratory but also at the point of care, but Dr. Sacks and coauthors noted that concern has been voiced about how reliable the POC instruments are for measuring βOHB concentrations. However, the issue, they said, is usually at concentrations greater than 5 mmol/L, "which is well in excess of any DKA diagnostic threshold."

"This issue might not only impact the identification of hyperketonemia but also, together with meter result imprecision, cause unreliable tracking in the rate of fall of BOHB concentrations," they added.

Point-of-care testing in the U.K. is common. In the U.S. it's difficult to determine the proportion of blood ketone measurements that are POC versus laboratory tests because the blood ketone meters are waived, Dr. Sacks and coauthors note. Point-of-care testing questions need to be resolved, Dr. Sacks tells CAP TODAY: "The evidence needs to be generated, and people need to do the studies."

Point-of-care testing is also an issue when measuring HbA1c.

One recommendation calls for restricting HbA1c POC testing for diabetes screening and diagnosis to FDA-approved devices at CLIA-certified laboratories that perform testing of moderate complexity or higher.

"The issue is that in the U.S., these devices are waived," Dr. Sacks says. "Numerous studies have shown that in general most point-of-care devices are not as accurate as those in the central lab, which should be no surprise to anybody."

No proficiency testing is required for the waived point-of-care HbA1c devices, "and a large component of the improvement in the quality of hemoglobin A1c testing has resulted from standardization," he says. The CAP plays an important role in standardizing and improving the precision of HbA1c testing because of the CAP Surveys, Dr. Sacks says, noting that the proficiency testing program for HbA1c is accuracy-based. "That has been one of the cornerstones of improvement."

For HbA1c testing, laboratories should be aware of potential interferences, including hemoglobin variants that may affect results depending on the method used. A new recommendation says assays of other glycated proteins, such as fructosamine or glycated albumin, may be used where abnormalities in red blood cell turnover, hemoglobin variants, or other interfering factors compromise the interpretation of HbA1c results. (See CAP TODAY, https://bit.ly/3ungGtl.)

"There's more and more evidence being generated from studies showing the value of these other markers—particularly glycated albumin—of chronic glycemia," Dr. Sacks says, "sometimes in conjunction with hemoglobin A1c but clearly in people for whom hemoglobin A1c cannot be used."

Glycated albumin or fructosamine measure the average blood glucose over the prior two to three weeks, which is far shorter than that of HbA1c, which reflects the prior eight to 12 weeks, he notes, but "does get rid of the minute-to-minute, literally, fluctuations in blood glucose." The principle is the same as for HbA1c, he says: "Hemoglobin A1c is hemoglobin that has glucose irreversibly stuck on; glycated albumin is albumin that has glucose stuck on." But albumin remains in the blood for only a few weeks, whereas red cells live for 120 days, so glycated albumin reflects the average glucose over a much shorter period.

A revised recommendation addresses the problem of glycolysis in sample collection tubes, which the authors write "will lead to missed diagnoses of diabetes in the large proportion of the population who have glucose

concentrations near the cutpoints for diagnosis of diabetes."

To minimize glycolysis, they recommend using a tube containing a rapidly effective glycolytic inhibitor, such as granulated citrate buffer, to collect the sample. If this cannot be achieved, the recommendation says, the sample tube should immediately be placed in an ice-water slurry and subjected to centrifugation to remove the cells within 15 to 30 minutes.

"We changed the recommendation to a citrate buffer to prevent glucose breakdown in test tubes," Dr. Sacks says. While these tubes are available in many European and other countries, they're not yet available in the United States. "I've been working with manufacturers for many, many years trying to get them to make these available in the U.S., and I am aware of one manufacturer that's planning to do this. They haven't applied for FDA approval," Dr. Sacks says, "but they're hoping to bring it up within the next few years."

The very low pH of the citrate buffer stops glycolysis immediately, he says. Sodium fluoride (2.5 mg fluoride/mL of blood, which is currently widely used to inhibit glycolysis) reduces glycolysis, but it still occurs over the first four hours after collection.

"If those [citrate] tubes become available, it will have a big effect, particularly in gestational diabetes because that's exclusively defined with a glucose tolerance test." Many hospitals wait until all the samples are collected. "But that means that in the first tube, the second tube, the glucose is going down" while the sample sits before it is sent to the laboratory, Dr. Sacks notes. Putting the tube on ice and centrifuging it within, ideally, 15 minutes is not practical for most hospitals, he says, adding that even double that time isn't practical. Tubes containing a citrate buffer will make the results much more consistent, he says. He and his coauthors call the decrease in glucose concentration in the sample due to glycolysis "a serious and underappreciated problem."

The prevalence of type 2 diabetes in women of reproductive age prompted a new recommendation related to gestational diabetes mellitus. It says all pregnant women with risk factors for diabetes should be tested for undiagnosed prediabetes and diabetes at the first prenatal visit using standard diagnostic criteria. "Several may have type 2 diabetes and not know," Dr. Sacks says. "It should be detected early." The guideline authors report that about 4.5 percent of women in this age group have diabetes, and 30 percent are unaware.

Also new is the recommendation that women with a history of gestational diabetes mellitus be screened for diabetes throughout their lives at least every three years using standard nonpregnant criteria. "People with GDM are at considerably increased risk of developing type 2 diabetes later," Dr. Sacks says. "Some people think of GDM as 'Pregnancy is a stress on the whole hormonal balance and glucose homeostasis. So it's unmasking borderline diabetes.'" Many women who are diagnosed with GDM develop type 2 diabetes in later years, despite it resolving post-pregnancy.

The number of recommendations related to continuous glucose monitoring (CGM) doubled from four to eight.

The devices were fairly new in 2011 and are now widespread, "and the evidence for their use is much stronger—there are more situations where they're recommended," Dr. Sacks says.

Dr. Sacks

For laboratory staff, the connection with continuous glucose monitoring is that point-of-care testing may be

performed in a patient who comes into the hospital with a monitor. “Then you have to make a decision whether to allow the person to use their CGM to select the insulin dose or whether the nurses do point-of-care glucose testing, and that’s a hotly debated topic,” Dr. Sacks says.

“Some hospitals can do them together,” he says. “Each hospital has to have a committee that has to decide how they’re going to manage this because there is nothing official.” (CGM raises other questions for clinical laboratories. See “Disruptive technologies at the point of care,” https://bit.ly/3R9ceqV.)

On the topic of genetic markers, one of the three recommendations says that for selected diabetes syndromes, including neonatal diabetes and maturity-onset diabetes of the young (MODY), valuable information including treatment options can be obtained with definition of diabetes-associated mutations.

(The other two recommendations, largely unchanged from 2011, are that routine determination of genetic markers is of no value at this time for diagnosing and managing type 1, and there is no role for routine genetic testing in people with type 2.)

Published in the same issue of *Clinical Chemistry* is an editorial by Andrew Hattersley, professor, University of Exeter Medical School, who writes that “it is disappointing that the guideline stops short of giving comprehensive guidance on laboratory testing for monogenic diabetes.” Molecular genetic testing guidance is needed, he writes, on who should be tested, how they should be tested, what to test, and how to interpret the results.

Of the recommendation to test for recognizable clinical syndromes, he says MODY is rarely recognized clinically. “A better approach proposed in the ADA diagnostic guidelines is to consider MODY when patients do not have typical characteristics of type 1 or type 2 diabetes,” he writes, “particularly when there is evidence of dominantly inherited diabetes in a family.” The key laboratory characteristics that define typical characteristics of type 1 diabetes are the presence of multiple islet autoantibodies at diagnosis and finding a very low or undetectable C-peptide in long-duration patients. “Finding one of these laboratory findings can effectively exclude the need for genetic testing in the vast majority of children or young adults,” he writes.

Regarding how to test, Dr. Hattersley says the guideline makes no mention of next-generation sequencing, and yet: “With current NGS, there is minimal cost in including all robustly defined monogenic diabetes genes in a single test.”

With further reductions in the cost of testing, he writes, monogenic testing in diabetes will become increasingly available.

In a separate editorial, on the topic of the third edition of the lab analysis guidelines and recommendations overall, Eric Kilpatrick, MB, ChB, of the Division of Clinical Biochemistry, Sidra Medicine, Doha, Qatar, and Weill Cornell Medicine-Qatar, writes the “authors are to be congratulated on what they have achieved with this latest edition and once again show how the close relationship between specialists in laboratory medicine and clinical diabetes can be of direct benefit to patients.”

Some of the clinical progress made in treating diabetes, Dr. Kilpatrick writes, “would at best have been delayed were it not for the role of laboratory diagnostics.”

Amy Carpenter is CAP TODAY *senior editor.*