Another Step Toward Standardization of Methods for Measuring Hemoglobin A₁c

In this issue, Kobold et al. describe an exciting new Reference Method for hemoglobin A₁c developed by the International Federation of Clinical Chemistry Working Group on Hemoglobin A₁c. To our knowledge, this method represents the first time mass spectrometry has been applied to a protein of clinical interest in a high-level (Definitive) Reference Method. Measurements of hemoglobin A₁c (or, alternatively, of glycohemoglobin) are increasingly important in the monitoring of glucose control in patients with diabetes. The test’s importance became especially prominent after the publication of the Diabetes Control and Complications Trial (DCCT), which demonstrated the importance of control of blood glucose in the prevention or delay of the complications of diabetes. The test has recently caught the eye of the US government as one that is underutilized. Thus, it is not much surprise that both an AACC Subcommittee, which has evolved into the National Glycohemoglobin Standardization Program (NGSP), and an IFCC Working Group, from which Kobold’s report emanates, have been addressing glycohemoglobin standardization over the past several years.

Several other editorials in this Journal have commented on important papers relating to glycohemoglobin over the years. Because the most recent of these, one by Miedema, gave such a good overview of the chemistry of glucose addition to hemoglobin, nomenclature, and analytical methodologies, we will not repeat this information here. Instead, our discussion will focus on the relative importance of accurate results, harmonious results, and the preservation of reference values with which clinicians are familiar.

The application of knowledge gained in any large clinical research study to routine medical practice is frequently a challenge. Of particular interest to clinical laboratorians are studies in which major therapeutic decisions are based on a clinical laboratory result. The scale used to measure the concentration of a brand new analyte within a single laboratory is relatively unimportant—laboratories merely establish their own reference values and do not worry about interlaboratory comparability. To some extent, laboratory-specific reference ranges can compensate for lack of interlaboratory agreement. However, with patients becoming increasingly mobile, often being seen in multiple healthcare facilities in the same week, and with physicians trying to apply published research study laboratory data to day-to-day clinical decisions, there really must be some guarantee of comparability of laboratory results.

This need for comparability, often termed “harmonization,” of clinical laboratory results is the foundation of what have been called traceability systems. In metrological terminology, a valid traceability system must have an unbroken chain of comparisons, each with a defined degree of uncertainty, all the way from the day-to-day clinical laboratory results to the “highest level” Reference Method. In the US National Reference System for Clinical Laboratories (NRSCL), the highest level Reference Methods are called Definitive Methods. Usually, one or more intermediate-level Reference Methods, which are easier to perform and more widely available, are in use to facilitate traceability back to the highest level (Definitive) Reference Method. Occasionally, no methods are available that qualify as a Definitive or Reference Method, in which case one of the “best available” clinical methods is selected to begin the harmonization process. Within the NRSCL, such a method is called a “designated comparison method,” a term that Kobold et al. use to describe the DCCT study’s BioRex 70-based Reference Method. This BioRex 70 method had been the Reference Method for the DCCT study and now forms the basis for the NGSP.

Besides intermediate- and high-level Reference Methods, the other key components for truly functional reference systems are commutable reference materials with long-term stability. Such materials are typically lyophilized or frozen reference serum, plasma, or whole-blood preparations. While long-term stability is usually not too difficult to achieve, problems with nonuniform recovery of the analyte of interest by a wide variety of clinical methods is often a problem. Examples of two mature reference systems are those for glucose and cholesterol. Both have high-level Reference Methods, available in only a few laboratories worldwide, that are based on isotope-dilution mass spectrometry (ID/MS) at the top of the traceability chain, as well as intermediate-level Reference Methods (e.g., Abell–Kendall and hexokinase methods, respectively). Unfortunately, the reference system for glycohemoglobin is not nearly so advanced, largely because it has not had the benefit of nearly a half-century of refinement, which the cholesterol and glucose reference systems have enjoyed.

Even with cholesterol and glucose, appropriate reference materials have been a problem. For cholesterol, only recently has a highly commutable frozen serum reference material without any additives or stabilizers been produced. For glucose, the US National Institute of Standards and Technology recently released a frozen serum-based glucose reference to correct a significant problem with glucose stability in many current lyophilized reference materials. Lyophilized reference materials for glycohemoglobin have suffered from noncommutability, giving quite different results by various analytical
methods [11]. Deep-frozen hemolysates appear to work well for some glycohemoglobin methods, but not all. Weykamp’s group has worked hard on improving the lyophilization process to produce a widely commutable material, but the degree to which this material works with all the widely available clinical methods is still somewhat uncertain. All in all, we believe a stable reference material still needs some work and validation.

There seems to be no question that the results of the IFCC GC/MS method are more nearly accurate than those of previous methods. It is now well documented that results of the BioRex 70 “reference method” include other hemoglobin adducts, such as carbamylated and acetylated hemoglobin. Consequently, its mean value in nondiabetic individuals is ~5.2%, notably greater than seen with higher-resolution HPLC methods (e.g., polyCAT A and Mono S), which yield mean values closer to 3.4% in nondiabetic individuals. According to Fig. 7 of Kobold et al., presumably the mean value in nondiabetic subjects with the new IFCC method would be even a few tenths of a percent below the polyCAT A value.

The key clinical questions are, what will and what should happen to the current efforts to standardize clinical glycohemoglobin results to a clinically interpretable frame of reference? We foresee two possibilities. One is analogous to what happens with cholesterol. Most major clinical studies (e.g., the Framingham Heart Study, the Coronary Primary Prevention Trial) traced their analyses to the Abell–Kendall Reference Method. The National Cholesterol Education Program developed an extensive system for tracing clinical laboratory methods to this reference point and made major efforts to train clinicians to interpret and act on values based on cut points defined by the Abell–Kendall assay. Although ID/MS methods became available that showed the Abell–Kendall value was slightly higher than the ID/MS values—as with glycohemoglobin, because of analytical nonspecificity [12]—the decision was made, at least in the US, to continue to calibrate clinical methods by the Abell–Kendall values. The ID/MS methods merely serve as a secondary anchor to periodically validate and confirm Abell–Kendall results for reference materials. However, bias between the ID/MS method and the Abell–Kendall method is only a few percent, not the nearly 40% the IFCC mass spectrometry method appears to have relative to the DCCT BioRex 70 method. If the IFCC mass spectrometry method is used only as an anchor for periodic validation of the BioRex 70 method, some difficulties are likely to result from the fact that both the slope and the intercept of this comparison are nonzero.

The other possibility for handling results of the new IFCC Reference Method seems to be to proceed as in the case of glucose. Early clinical assays, such as the ferricyanide method, which were still in widespread use when we were in training, had substantial analytical interferences from nonglucose reducing substances. As analytically more-specific enzymatic glucose methods were introduced clinically on a widespread basis, reference values for glucose concentration in fasting and glucose tolerance test situations simply fell. Clinicians seemed to relearn clinical reference intervals (then called “normal ranges”) and clinical decision points as the methods became analytically more specific. Perhaps a similar transition in reference ranges will happen with glycohemoglobin. However, we note two caveats. First, the switch from whole blood to plasma or serum glucose as the most frequent clinical specimen was occurring simultaneously with the introduction of enzymatic glucose methods in clinical laboratories. This switch in specimen type had as much or more impact on reference ranges and decision points than did the elimination of the analytical nonspecificity of the early reducing methods. Second, the highly specific enzymatic methods now form the analytical basis for the vast majority of the clinical methods. If consistency with the “old” reducing method for glucose results were to be maintained, it would mean converting highly accurate results to results that were biased high, merely to maintain long-term temporal stability. By contrast, few current glycohemoglobin methods yield results that are interchangeable with results of the IFCC method.

What will happen with glycohemoglobin measurements remains to be seen. Will clinicians need to be retrained to expect lower values in healthy, nondiabetic individuals and in comparable states of long-term glycemic control than were found in the DCCT study reports, or will DCCT-based values continue to be widely used clinically as is now being promoted by the NGSP in the US? What exactly is hemoglobin A1c, which was originally a term for an ion-exchange chromatographic peak, but now is being defined by the IFCC Working Group as hemoglobin molecules that are irreversibly glycated at one or both N-terminal valines of the beta chains? What is the best thing to measure clinically? For example, some experienced diabetes researchers have suggested that affinity methods, which to a large extent measure glycohemoglobin regardless of the glycation site, may actually be more useful clinically in reflecting glycemic control (F.Q. Nuttall, personal communication), but it is the BioRex 70-defined glycohemoglobin that is accompanied by the vast amount of clinical data from the DCCT.

Whatever the outcome, very clear communication with clinicians must be of the highest priority while analytical methods continue to evolve and harmonization remains a goal. In 1992, one of us (D.E.B.) predicted that we might see the turn of the century before we saw a Reference Method and reference materials and a reference system applied in clinical laboratories in measurements of this important marker for diabetic control [5]. Now, less than 30 months from the year 2000, the work of Kobold et al. brings us an important step closer to the goal, a goal that remains important in the care of people with diabetes.
References


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