

Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Measurements of glucose are being used worldwide to diagnose diabetes, and to identify patients at risk of developing diabetes. Accurate measurements of glucose are becoming increasingly critical. Decisions are being made at lower concentration seen in large numbers of people worldwide, and even small errors may lead to the misclassification of many patients.

Dr. David Bruns is a co-author of an editorial from the May issue of *Clinical Chemistry* that reports on this topic. He is a Professor of Pathology, Director of Clinical Chemistry and Toxicology, and Associate Director of Molecular Diagnostics Laboratory at the University of Virginia. He is also our guest in this podcast.

Dr. Bruns, we have been measuring glucose forever. How can it be that there are still issues regarding measurement standards?

Dr. David Bruns: Yes, you are absolutely correct. Glucose has been measured since antiquity. Dr. David Sacks, a colleague of ours, likes to point that early measurement of glucose in urine were, that we might say bioassays. For example, flies were attracted to urine with high sugar contents. So that was a tip off that there was glucose in the urine. And similarly physicians used their taste buds to detect sweetness in urine. Fortunately for me the latter method was superseded by chemical methods before I started medical school. But the despite the long history there are still problems.

Host: Now, aren't glucose assay standardized?

Dr. David Bruns: Well yes, it's absolutely true. In central laboratories, the assays are standardized, and they are quite well standardized, and not only standardized, but they are quite precise. We have good data to show that variation in measurement of glucose among laboratories is really quite small.

On the other hand, the standardization has not been done for glucose meters. The standardization of meters is complicated by the lack of a reference method and of a certified reference materials for glucose in whole blood samples, which is what glucose meters must do use of course.

Host: Well, if central lab methods are standardized and only central lab methods are used for diagnosis of diabetes, then where is the problem?

Dr. David Bruns: Much of the problem in the diagnosis of diabetes when it is based on glucose measurements, stems from the handling of samples, from the time the blood sample is drawn from the patient until it gets to the laboratory.

In the studies that defined the cut points for diagnosis of diabetes, sample handling was usually done very carefully, so that there was no loss of glucose by glycolysis between the time the sample was drawn, and the time the analysis was performed. Although there is a considerable amount of variation in how well the samples were — or how carefully the samples were handled in the studies that defined the diagnostic criteria for diabetes based upon blood sugar measurements.

Now the way samples are handled in real life can be different. Let me just give one quick example. In the recent HAPO study performed in many centers around the world with tens and thousands of pregnant women to define the cut point for gestational diabetes. Samples were collected and immediately placed in an ice slurry, which stops glycolysis completely.

And so, the cut points that had been established for — that I think will be shortly established for diagnosis of gestational diabetes based upon these studies, really reflect as close as one can hope to find the patient's true blood sugar at the time that the sample was drawn.

Of course, in the real world by the time we get a sample in the laboratory, even if it has been collected with sodium fluoride, we have probably lost anywhere from 5 to 15% of the glucose in the sample to glycolysis. The sodium fluoride as others have shown has no effect during the first hour or two after the blood sample is collected. So sodium fluoride doesn't solve the problem.

So the values that we will get in the samples from pregnant women are really going to be biased. To further complicate this situation in the real world, sample handling in changing and samples are getting to the laboratory faster, so that this is leading to higher measured concentrations of glucose, than we had in the past.

So all of those factors I think are combined, as well as some others that I haven't mentioned, that lead to problems because of pre-analytical handling of samples.

Host: Well, in your opinion can this be fixed and how?

Dr. David Bruns: Well, that's our challenge. I think one thing, and first of all I think we need to determine the relationship of current

sample handling techniques and those used in the studies that defined the cut points for diagnosis of diabetes. Those studies are very difficult to do, they are very long term studies, because the way those studies have been done and correctly, is to measure glucose in patients, in apparently healthy people at baseline, and determine at what levels of fasting glucose are glucose tolerance test values. We start to find that patients develop over time the complications associated with diabetes. Those are very difficult studies and take a lot of time, so it's difficult to redo them.

So we would like to know how the samples were handled in those exact studies. One thought is to modify cut points to reflect current sample handling techniques. This will lead to higher cut points and thus fewer people would be diagnosed with diabetes. I personally am leery of such an approach.

If we think of the example of gestational diabetes in recent HAPO study, we could think in terms of how can we make our routine methods give results that would be equivalent to those that were obtained in the HAPO study where samples were put on ice.

One approach is to ensure very rapid centrifugation of the blood samples to remove the plasma from the red cells and white cells that are responsible for the glycolysis. And if that can be done very rapidly and the plasma is kept separate from the cells, either by a barrier or by simply removing it through a separate tube we'd be okay. Another possibility at least for the gestational diabetes patients, would be to handle all the samples on ice, again, not as appealing.

A third alternative in the gestational diabetes scenario, it appears to be the type of blood collection tube described in the article by Gambino and his colleagues, which is the article that our editorial was about. This acid citrate tube appears to stop glycolysis completely and essentially immediately.

So the results with that tube should be equivalent to results obtained from a tube of blood that is immediately placed in ice and handled at low temperature until the plasma is separated from the cells. Unfortunately that tube type is not available to my knowledge yet in the United States, but if that could be arranged, I think we would have a solution for gestational diabetes situation or scenario.

Now whether that would be appropriate for other situations in adults who are not pregnant, where the cuts points for diagnosis of diabetes were established not with the samples that were on ice, but rather with samples closer to the real world of current clinical practice, it's probably not going to work as well there.

I think you can see there are a number of options and a number of hurdles that we are struggling with at this point.

Host: Well that addresses the central lab issues, but what about the meters? We know they are not good enough for central lab methods, but aren't they good enough for what they were designed for?

Dr. David Bruns: That's the crux of the issue, is whether they are good enough for their purpose. It's not a question of is the CV less than some number or is the bias less than some other number, the question is, are they good enough for their medical application?

Meters are cleared by the FDA for use in home monitoring of blood glucose and adjustment of therapy, including adjustment of insulin dose. But no one can really tell the FDA how much imprecision or bias is acceptable and will not lead to errors in the insulin dose. And even more importantly, we do not know how much meter error can be tolerated without impairing the patient's ability to achieve the desired level of glucose control, and critically to avoid hypoglycemia.

One could even add, we certainly don't know how much error can be tolerated before there is some degradation in outcomes such as mortality and morbidity. This I think is what we need to find out.

We do know from careful studies that patients who use meters to help lower their average blood glucose had fewer complications of diabetes than did patients in a control group, who were not using meters to lower their average blood glucose.

This finding though doesn't allow a conclusion that the meters used in the study were adequate for their purpose, and that any meter with similar characteristics is acceptable. Actually some years ago Dr. Jim Boyd and I did simulation modeling studies that showed that a large percentage of insulin doses will be in error with current meters.

We do not know if improvement of the meters will lead to improved patient outcomes, but we can I think predict with some confidence that improved meter performance, in other words improved precision or less imprecision, and less bias will decrease the percentage of insulin doses that are in error, if the patient is using the kind of insulin dosing scheme that was modeled in the computer.

Finally, another use of these meters is for a tight glucose control in intensive care units. Of course, this is an off-label

use of meters, as none of the meters are cleared by the FDA for this use.

Host: Now I have recently that tight glucose control in ICUs is dangerous. Does the measurement of glucose have a role in this?

Dr. David Bruns: Well, I believe that it does. The study you have heard of, I assume if you want to call it Nice Sugar. Nice Sugar was done as a multinational study, Australia, New Zealand, US, and, I believe, Canada, in which a variety of meters were used, a variety of ways to measure the glucose in the ICU, and in all the different centers the same algorithm was used to adjust insulin doses.

You can imagine that was a wide variety of meters and other methods that were used to measure glucose, some of them measured too low, some of them had bias that was low, some had bias that was high, and from published studies of meters we know that those biases can easily be more than 10% in either direction.

Obviously, if you have meters that are 10% high and 10% low, and yet you are using the same algorithm for all patients to decide on insulin dose, you will have some patients whose blood sugars will be although measured in the right target range will wind up being much lower than others and some will be much higher.

And I think that this helps to explain some of the reasons that in that trial there was actually a higher mortality rate than in the control group. Some of the patients became the hypoglycemic, and I think that's predictable. And of course, hypoglycemia would not be recognized if the meter was biased high.

So that's one issue with regard to the Nice Sugar study that received quite a bit of publicity recently. Now another way of looking at this is again to do some simulation studies, and again, we have done some preliminary simulation studies of tight glucose control and the studies so far, the preliminary studies show so far that meter imprecision markedly impairs the ability to hold blood glucose concentrations in the desired ranges, the target ranges.

And this is really not very surprising, given that the range of measurement error with meters may be as large as the target range for the glucose concentrations in the patients. And then of course meter bias, which I have already mentioned, also makes it impossible to achieve and maintain desired concentrations of glucose in the patients, and we hope to extend these preliminary modeling studies by incorporating a powerful model of glucose homeostasis in

the model. Using this homeostasis model that was developed by the University of Virginia, and when that is incorporated, I believe the model will allow confident quantitative predictions about the magnitude of the impact on patients of meter errors. We are currently looking for funding to support this work, and hope we will be able to get that and do the work over the course of the next year or so.

Host:

Dr. David Bruns is a professor of Pathology, Director of Clinical Chemistry and Toxicology and Associate Director of Molecular Diagnostics Laboratory at the University of Virginia. He has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.

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