Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which patients develop hyperglycemia. Type 1 diabetes results from cellular-mediated autoimmune destruction of the insulin-producing pancreatic β-cells (1). Patients with type 1 diabetes frequently present with acute symptoms, including life-threatening ketoacidosis, and all patients require lifelong insulin therapy. Type 2 diabetes, which accounts for approximately 90% of all diabetes, has an insidious onset due to combined insulin resistance and insufficient insulin production. In many patients, type 2 diabetes can be controlled with diet, exercise, and oral agents. The worldwide incidence of diabetes has been increasing dramatically. Although most of the rise has been for type 2 diabetes, type 1 has climbed by 2.8% to 4.0% per annum, rising 21% in US youth between 2001 and 2009.

At disease onset, most type 1 diabetes patients have autoantibodies to 1 or more of the following: islet cell cytoplasm, insulin [insulin autoantibodies (IAAs)] (2), the 65-kDa form of glutamic acid decarboxylase (GAD65), insulinoma-associated antigen 2 (IA-2), and zinc transporter 8 (ZnT8) (these are collectively termed islet autoantibodies). Moreover, the presence of islet autoantibodies predicts the development of type 1 diabetes. A recent prospective study measured IAA, GAD65, and IA-2 in 13,377 children who were at high risk for type 1 diabetes and followed them for 15 years (2). Virtually all the children who had multiple islet autoantibodies developed type 1 diabetes, whereas approximately 15% with a single islet autoantibody progressed to type 1 diabetes.

Quantification of islet autoantibodies has proved difficult. Islet cell antibodies were the first autoantibodies identified in type 1 diabetes and are measured on frozen sections of human pancreas by indirect immunofluorescence. The method is technically demanding, labor intensive, difficult to standardize, and currently performed by few laboratories. The other antibodies are most frequently measured by RIA. Recently, nonradioactive ELISA methods have become available commercially for GAD65, IA-2, and ZnT8 (the last was approved in September 2014 by the US Food and Drug Administration for use in the US). Nevertheless, RIA remains the most widely used technique for patient samples and clinical studies. Considerable effort has been undertaken to standardize autoantibody assays. The Centers for Disease Control and Prevention and the Immunology of Diabetes Society established a proficiency testing program, Diabetes Antibody Standardization Program (DASP), in 2000. The goals of DASP are to improve laboratory methods, evaluate laboratory performance, support the development of sensitive and specific measurement technologies, and develop reference methods (http://www.cdc.gov/labstandards/diabetes_dasp.html). The Islet Autoantibody Standardization Program has replaced DASP. Participating laboratories (approximately 50 laboratories in approximately 20 countries) analyze autoantibodies in serum from 50 patients with newly diagnosed type 1 diabetes and 100 controls (3). These interlaboratory comparisons have substantially reduced variation among laboratories. Nevertheless, there remains considerable room for improvement.

A recent paper in *Nature Medicine* (4) described a sophisticated plasmonic chip for diagnosis of type 1 diabetes via near-infrared fluorescence-enhanced (NIR-FE) detection of islet cell–targeting autoantibodies. The plasmonic chip comprises a 25- by 75-mm glass slide that has an irregular surface pattern of tortuous gold islands separated by approximately 10-nm gaps. The gold islands are functionalized with a thiol reagent to introduce carboxyl groups, and these form the attachment points for a layer of biocompatible branched polyethylene glycol (PEG). The PEG layer is functionalized with N-hydroxysuccinimide groups for attachment of islet-specific antigens (IAA, GAD65, and IA2). These are printed onto the activated PEG surface as an ordered microarray with approximately 400-nm-diameter features. The assay uses 2 μL of blood obtained from a finger stick. The blood is diluted 1:10 and applied to the array. Bound autoantibody is detected with a fluorescent IRDye800-conjugated antihuman IgG antibody [simultaneous detection of IgG, IgA, and IgM antibodies was achieved with a combination of cyanine 3 (Cy3), IRDye800, and Cy5 labels, respectively]. Bound dye is then detected with a NIR scanner. The entire assay can be
completed in <2 h. All test and control areas are printed in triplicate, and each plasmonic chip has a technical positive control (human IgG), a biological positive control (tetanus toxoid, which relies on positivity owing to the US tetanus immunization practice), and a negative control (phosphate-buffered saline).

In most analytical array-based assays, the solid support is passive, i.e., it merely serves as an easily manipulated object onto which a sample or reagent can be attached via physical or chemical methods. In the plasmonic chip-based assay, the solid support has a dual role: it also serves to actively modulate signal generation. A key to the success of this assay is the NIR-FE provided by the gold islands underneath the PEG layer. The enhancement (approximately 100-fold) is a function of the size of the metal nanostructures and is due to local increases in the electromagnetic field accompanying excitation of surface plasmon resonances in the gold, leading to enhanced radiative decay rates or fluorescence quantum yield. Other multiplexed immunoassays have been successfully developed on this type of plasmonic chip (e.g., cytokines and a 32-autoantigen panel), and as such, this technology may have broader applications to other diagnostic tests that require a multiplexed assay.

Several caveats to the study need to be considered. The number of patients enrolled was low, with only 39 individuals with new-onset diabetes (26 with type 1 and 13 with type 2) and 5 nondiabetic controls (4). The authors did not provide the criteria used to diagnose diabetes, but state that type 1 diabetes was established by “a positive signal in any of the 3 autoantibodies” they measured (4). This approach introduces considerable bias and is the reason they report a diagnostic sensitivity in their study of 100%, which is higher than in the published literature. It is generally accepted at the time of initial detection of fasting hyperglycemia that between 85% and 90% of individuals with type 1 diabetes will have islet autoantibodies (3). A limitation of the assay is that ZnT8 was not included. This is an important autoantibody detected in 60%–80% of newly diagnosed type 1 diabetes patients and in approximately 26% of patients negative for other islet autoantibodies (5). It is hoped that ZnT8 will be added to future versions of the chip. Perhaps most importantly, the practical value of testing remains contentious, and there is considerable disagreement regarding the clinical utility of islet autoantibody measurement. Some experts advocate screening individuals at high risk for type 1 diabetes for autoantibodies to avoid delay in starting insulin therapy; others counter that autoantibody analysis is unnecessary because insulin is introduced when patients are hyperglycemic. No treatment has been identified that reliably prevents, cures, or delays the appearance of type 1 diabetes. Therefore, guidelines do not recommend using islet autoantibodies for screening or for routine diagnosis of diabetes (3).

They may be used for classification of diabetes in adults and may be considered in research studies. It is important to realize that 1%–2% of healthy individuals have a single islet autoantibody but are at very low risk of progression to type 1 diabetes. Coupled with the very low prevalence (approximately 0.3%) of type 1 diabetes, the positive predictive value of a single islet autoantibody is extremely low. The matter is further complicated by the wide variation in time interval between seroconversion and onset of disease, which ranges from weeks to 18 years (2). Another confounding factor is the HLA (major histocompatibility complex) system. HLA genes are integral components of the adaptive immune response and can be useful to indicate the absolute risk of type 1 diabetes. Some HLA genes are associated with increased susceptibility to type 1 diabetes, whereas individuals with other HLA genes are protected. Epidemiological studies have shown that the predictive value of a positive islet autoantibody is considerably diminished in the presence of protective HLA genes (3). Together, these factors make it difficult for a clinician to decide how to manage an asymptomatic individual with autoantibody positivity.

A particular claim for the present work is that it provides a point-of-care platform. Certainly, the ability to use a drop of whole blood taken from a finger stick is conducive to point-of-care applications, and the next step should be streamlining of the assay and miniaturization of the scanner to meet the goal of implementation in a primary care physician’s office. However, as yet, a compelling case has not been made for testing for autoantibodies at the point of care in the context of diabetes. Nevertheless, the Nature Medicine paper describes a novel approach to accurately quantify islet autoantibodies, which has been technically challenging. We anticipate expansion of plasmonic chip technology to incorporate measurement of a wider array of analytes in the future.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interests: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: D.B. Sacks, Clinical Chemistry, AACC. Consultant or Advisory Role: None declared.

References