

Beacon Biotechnology's Point of Care Type I Diabetes Screen

Christina L. Roark, Roberta Thimmig, Ted Cull, and Millard Cull

Beacon Biotechnology LLC, Bioscience Park Center, 12635 E. Montview Blvd., Aurora, CO 80045

Introduction

Type I diabetes mellitus (T1DM) is an autoimmune disease where the insulin-producing β cells in the pancreas are destroyed. Islet cell antibodies (ICA) in type 1 diabetes were first discovered in the 1970's and later characterized as antibodies directed against a series of islet autoantigens, including for specific patients an isoform of glutamic acid decarboxylase (GAD65) and a protein tyrosine phosphatase-like molecule (IA-2). After the discovery of ICA, autoantibodies to insulin (IAA) were also demonstrated. While autoantibodies have not been shown to directly play a role in the destruction of β cells in humans or mice, circulating autoantibodies provide useful pre-clinical markers for diabetic autoimmunity. In fact, autoantibodies precede the development of diabetes for many months or years, allowing prediction of overt disease and identification of high-risk patients. Prospective studies have shown that the presence of autoantibodies directed against two or more antigens is more strongly associated with the risk of diabetes than is a high titer of an autoantibody to any single antigen. For example, the risk for developing T1DM within 5 years increases to 50% if anti-GAD65 and anti-IA-2 antibodies are both present. Moreover, patients with high-risk human leukocyte antigen (HLA) genes, who also have anti-islet autoantibodies, are even more likely to develop T1DM.

The BrightSPOT Platform

The BrightSPOT platform is inexpensive and disposable and derives all its power from a USB port.

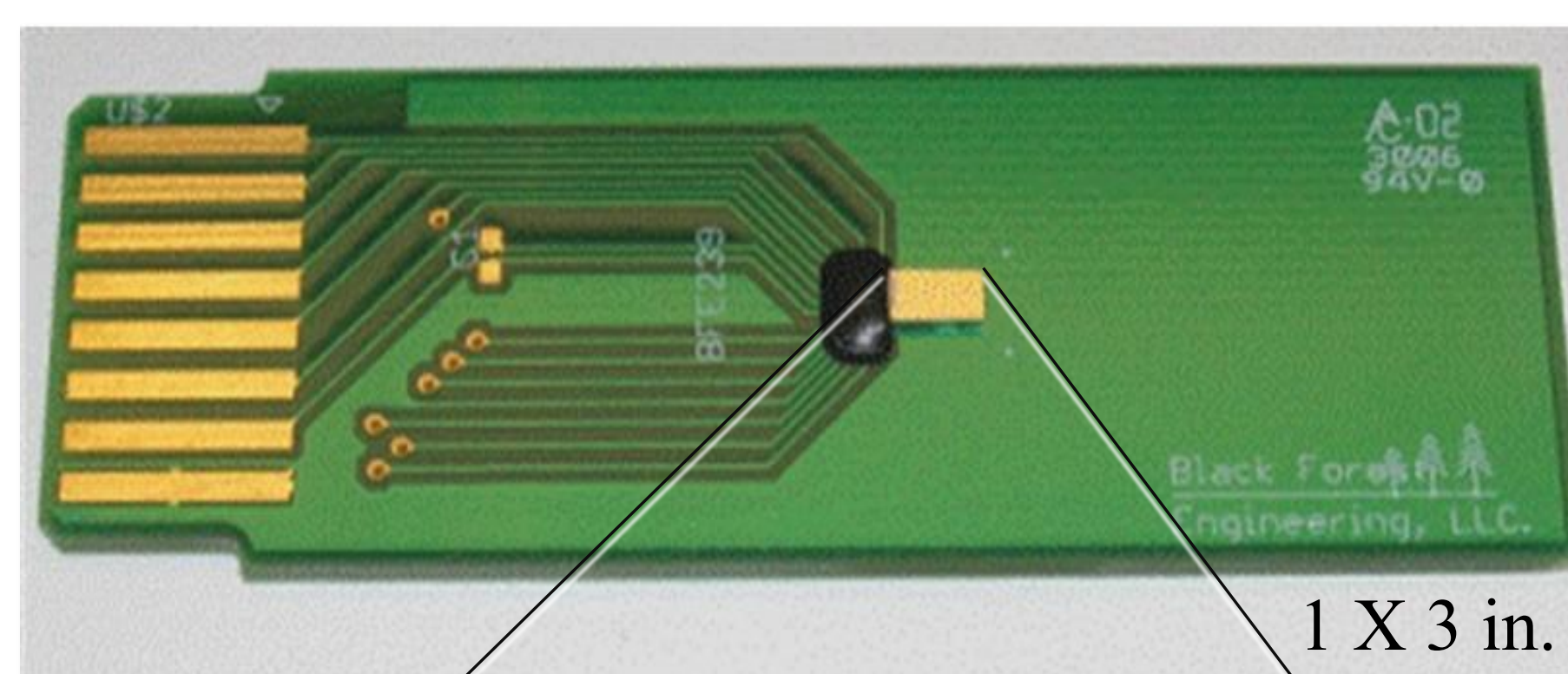


Figure 1. The BrightSPOT disposable device. The 5 x 2 mm light-detecting component is the rectangle in the center of the slide. The disposable device contains 112 individual and simultaneously addressable detector elements. Each can perform a different diagnostic test. The card edge (left side) interfaces with a USB adaptor.

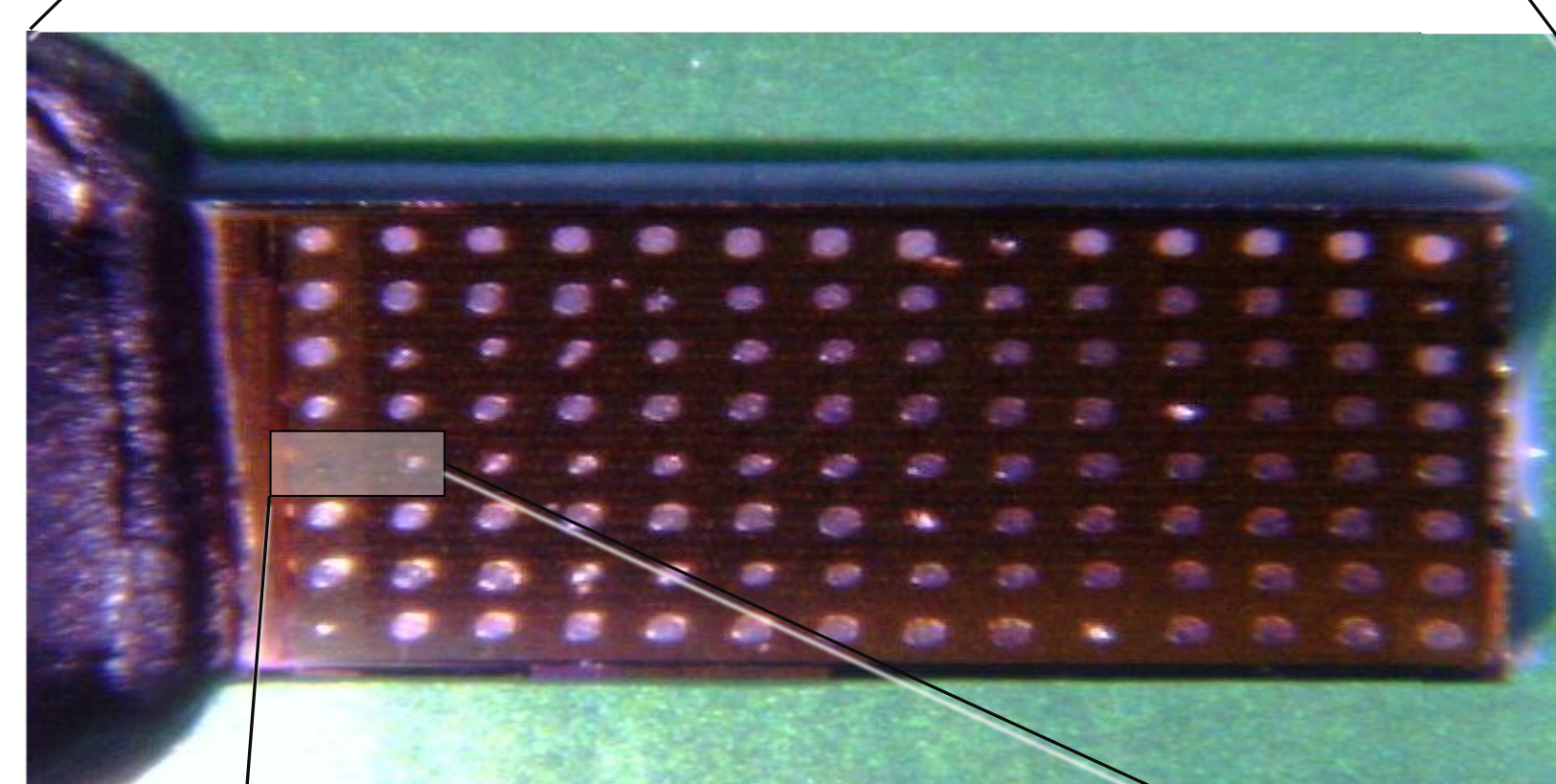


Figure 2. Magnified image of the 8 x 14 array of detectors spotted with 300 picoliters of biotinylated antigen (left).

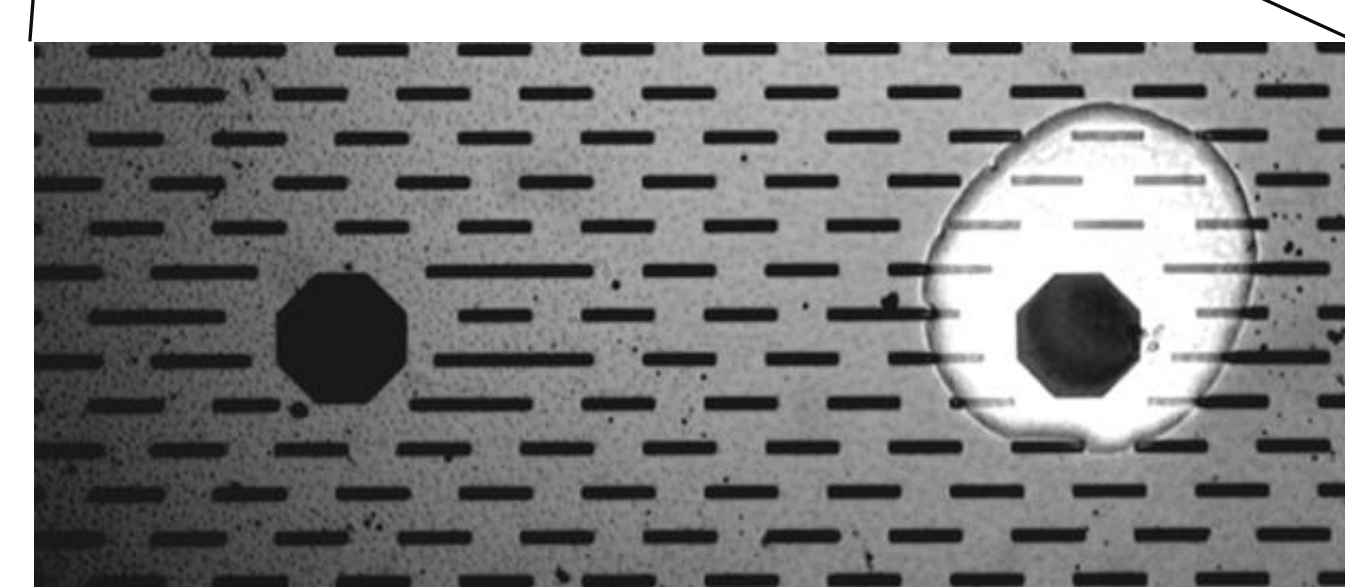


Figure 3. A higher magnification of figure 2, showing individual detectors - unspotted (left) and antigen spotted (right).

The 8 x 14 array on the BrightSPOT can be thought of as a miniature microtiter plate, each detector a one micron deep well that uses picoliter volumes of test reagents.

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Methods

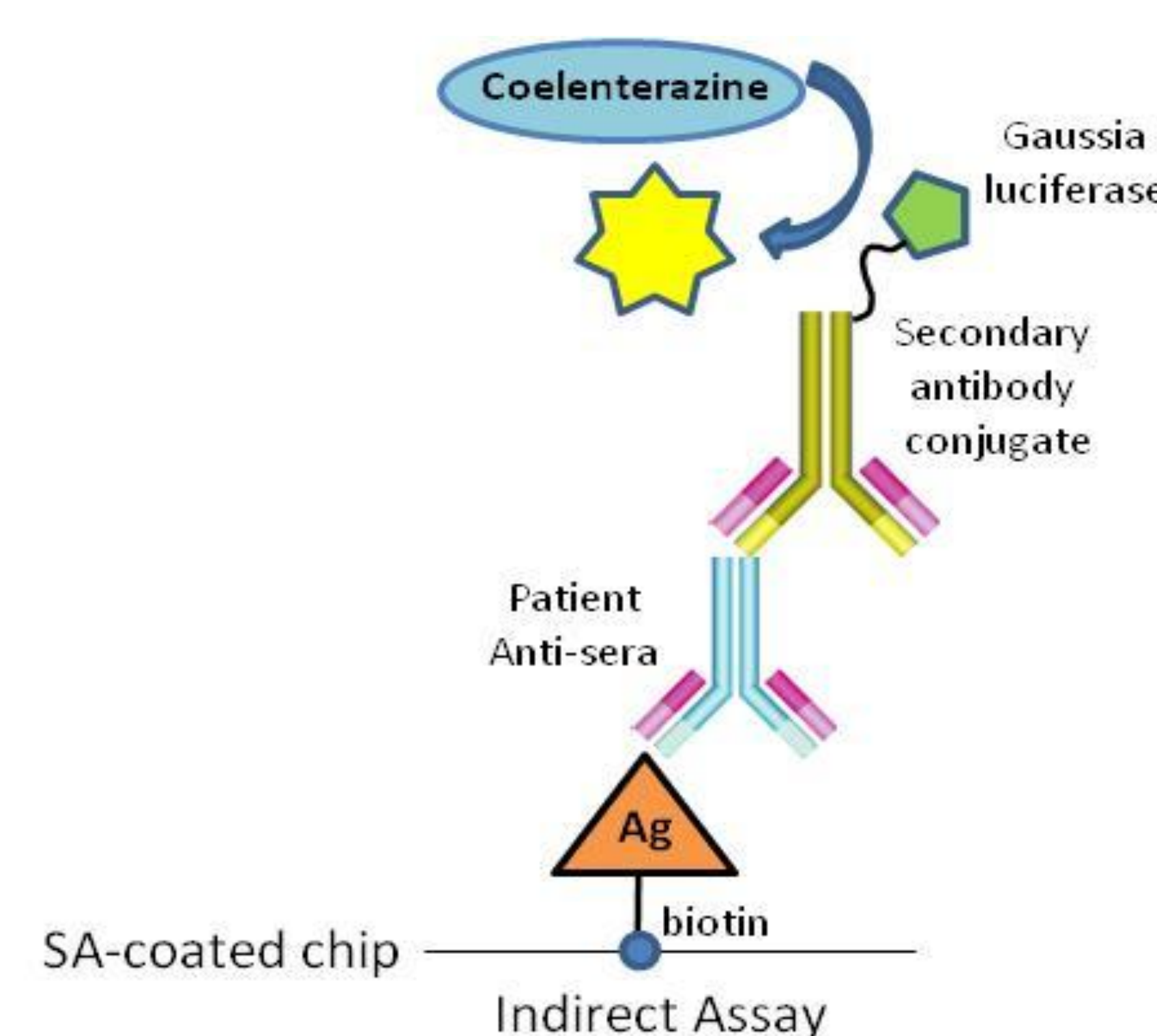


Figure 4. Schematic drawing of the indirect ELISA assay. The indirect ELISA uses biotinylated antigen on a SA-coated chip. Patient sera containing antibodies to the antigen are added and then detected using a secondary polyclonal antibody specific for human Ig that has been labeled with GLuc. When the substrate, coelenterazine, is added, light is generated.

300 picoliters of biotinylated GAD65 or normal human albumin was spotted onto individual pixels of a streptavidin-coated chip. The antigen-spotted chip was incubated with anti-GAD 65 spiked serum for 1 hour, washed, and then incubated with *Gaussia* luciferase-conjugated goat anti-human Fab fragments for 30 minutes. After washing, coelenterazine was added and the light measured. The individual detectors simultaneously measured the light produced by each test over a 60 second time-span, and the results were collected and tabulated by software on the computer.

Results

Anti-GAD65 antibodies can be detected in diabetic patient serum diluted 1 x 10⁶ fold using the BrightSPOT platform.

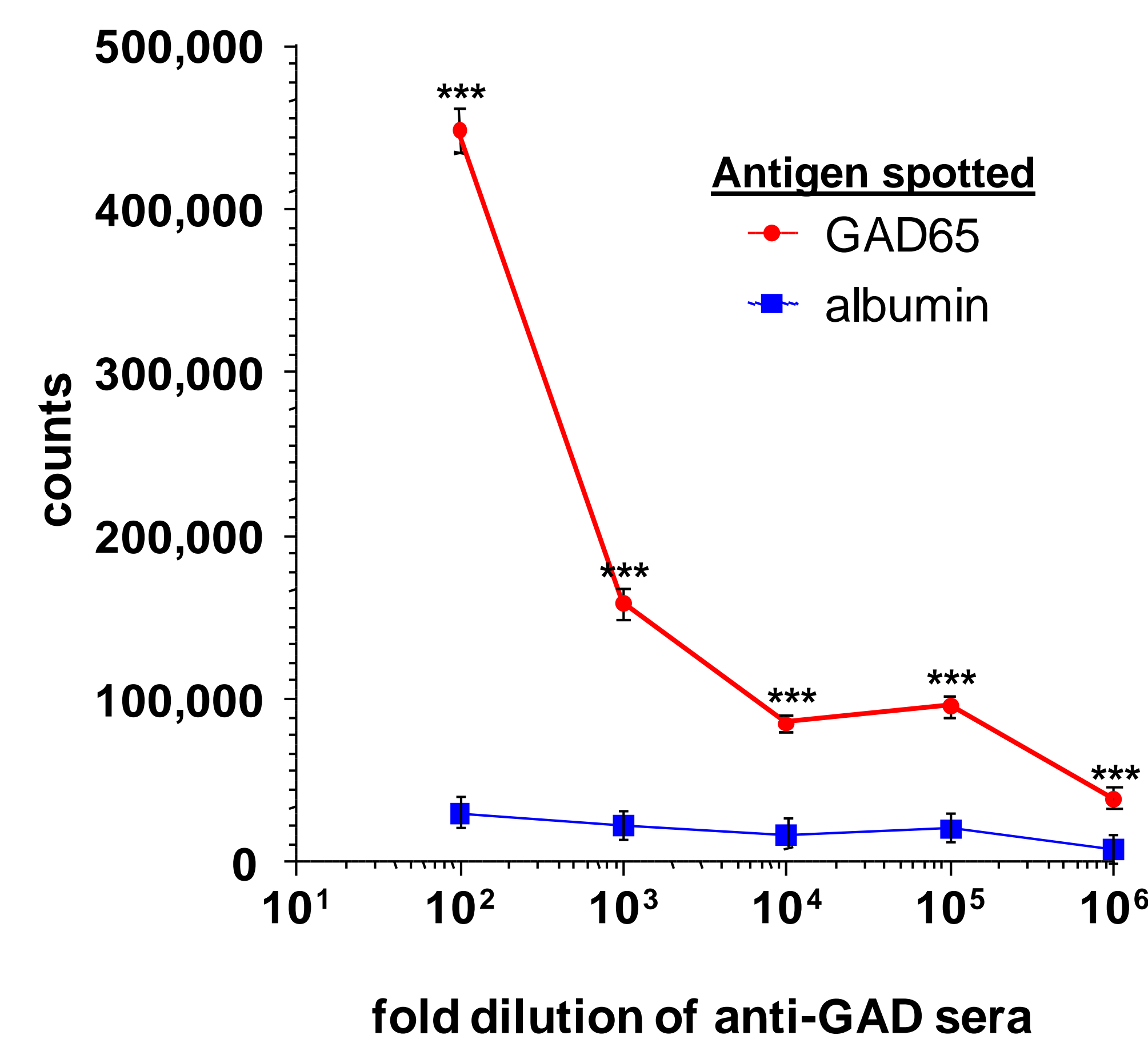


Figure 5. Biotinylated recombinant GAD65 and biotinylated human serum albumin (negative control) was spotted on a SA-chip. The light generated by incubating the chip with control serum (normal human serum) versus anti-GAD spiked sera was measured using the BrightSPOT platform as described in the text. Data shown is the average of 7 pixels +/- s.d. Unpaired t-test with Welch's correction was used to determine statistical significance for each dilution. ***P<0.0001

Results (cont.)

Anti-GAD antibodies are detectable in 5 μ l of whole human blood in less than 15 minutes using the BrightSPOT platform.

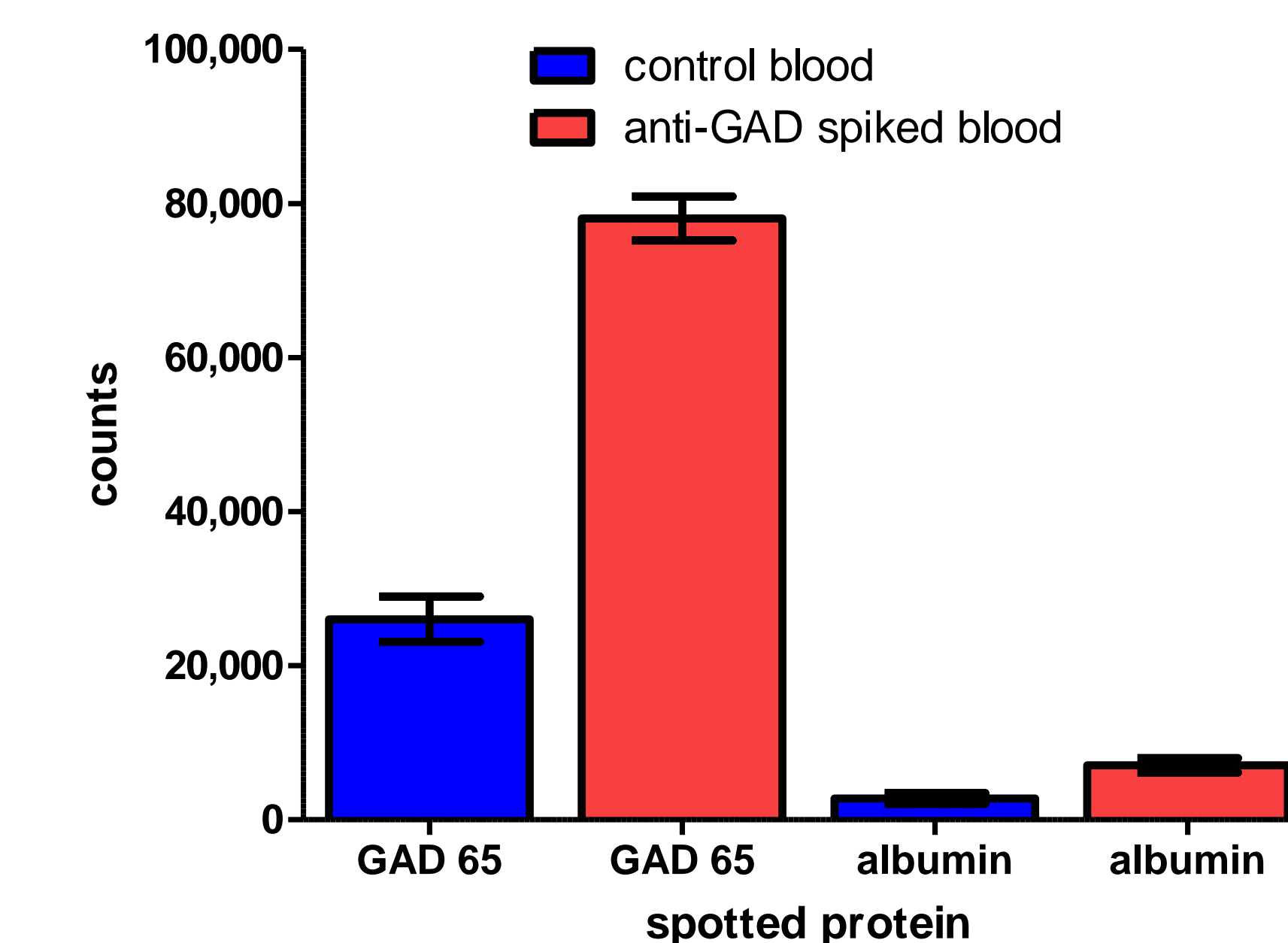


Figure 6. Biotinylated GAD65 and human serum albumin was spotted onto the chip and then after blocking, incubated with either 5 μ l of normal whole blood (control sera) or 5 μ l of anti-GAD spiked whole blood for 5 minutes as described above. After washing, GLuc-labeled anti-human Fab fragments were incubated on the chip for 5 minutes. coelenterazine was added to the chip and the light measured for 90 seconds. Data shown is the average of 4-7 pixels +/- s.d.

Summary

Table 1. Comparison of testing diabetic patient serum using a radioactive immunoassay (RIA) versus the BrightSPOT platform

	Limit of Sensitivity	Time to Result	Use in Resource-Limited Setting	Sample Prep	Radioactivity	Multiplexed
RIA	1:62,500 dilution	2 days	NO	serum	YES	NO
BrightSPOT	1:1,000,000 dilution	90 minutes	YES	whole blood	NO	YES

Conclusions

Beacon Biotechnology has begun developing a novel technology that allows for sensitive and rapid diagnostics at the point of care. We have shown that **anti-islet antibodies specific for GAD65 were measurable in patient serum diluted 1 x 10⁶ fold, which was 16 times more sensitive than a RIA assay. Importantly, the anti-GAD antibodies could be detected in a drop of blood in less than 15 minutes, indicating this technology may be useful for identifying patients at risk for developing T1DM.**

Acknowledgements

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