GYROS PROTEIN Technologies

Application Report 204

Insulin quantification: save sample and increase efficiency

- **Increased throughput (up to 112 data points in <60 minutes)**
- **Broad dynamic range**
- **Reduced cost per data point**
- **Excellent reproducibility**

Introduction

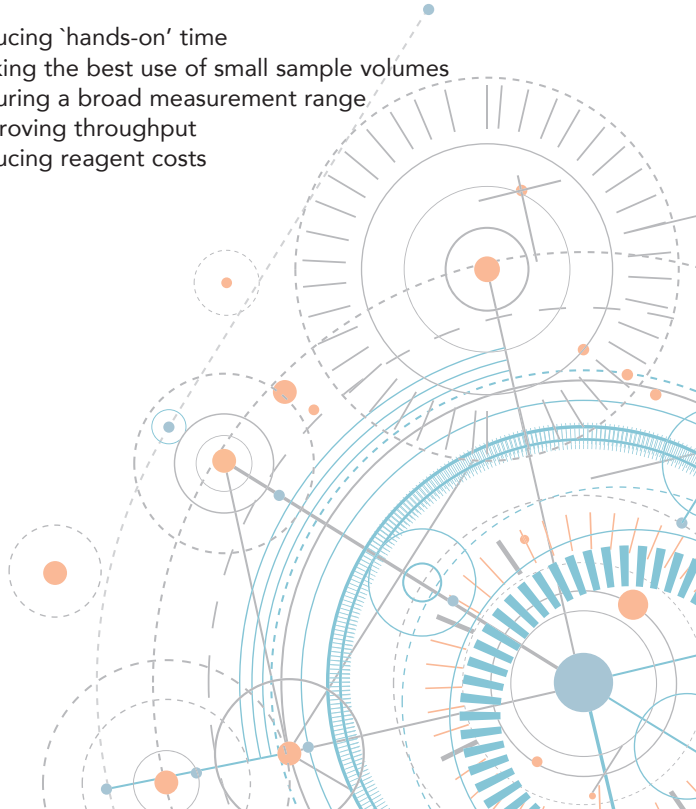
Diabetes, a disorder affecting insulin[1] production in the body, is increasingly common and research into its mechanisms and treatment is an ongoing area of investigation. There are two types of diabetes: Type I, where the beta cells produce insufficient amounts of insulin, and Type II, where the beta cells' capacity to release insulin is reduced. Insulin is, therefore, one of the most extensively studied metabolic factors in diabetes.

Current techniques for insulin quantification, such as ELISA and radioimmunoassays (RIA), have limitations in terms of assay performance and throughput. In particular, when using small animal models, where sample volumes are very small, and in patient screening and population studies, where large numbers of samples have to be analyzed, limited sample volumes and extensive hands-on time can become problematic. For such studies, conventional techniques require extensive 'hands-on' time and consume large quantities of reagents.

Immunoassays are among the most sensitive methods for protein quantification in biological fluids, and are widely used for animal and human trials in pharmacological research on different disorders.

In this Application Report, we present data from two collaborative projects with the Diabetes Center Karolinska, Karolinska Institute, investigating insulin production and NovoNordisk, developing an insulin analogue. Key issues for both groups include:

- reducing `hands-on' time
- making the best use of small sample volumes
- ensuring a broad measurement range
- improving throughput
- reducing reagent costs



Diabetes Center Karolinska

The research group at the Diabetes Center Karolinska, The Rolf Luft Center, Karolinska Institutet, Karolinska University Hospital is investigating the role of islets of Langerhans (cells that produce insulin) in Type I diabetes. Their research project involves extensive screening of patient material. The group measures insulin from human samples, mainly using radioimmunoassays (Grill et al. 1990), which involve extensive assay times. For measurement of rat and mouse samples, a commercially available ELISA kit[2] is predominantly used.

Limited sample availability is a key concern for the group whenever additional data is required, as when analyzing several analytes in one sample. As the research group would like to develop a method for measuring several metabolic factors per animal, maximizing the information from limited sample volumes is very important.

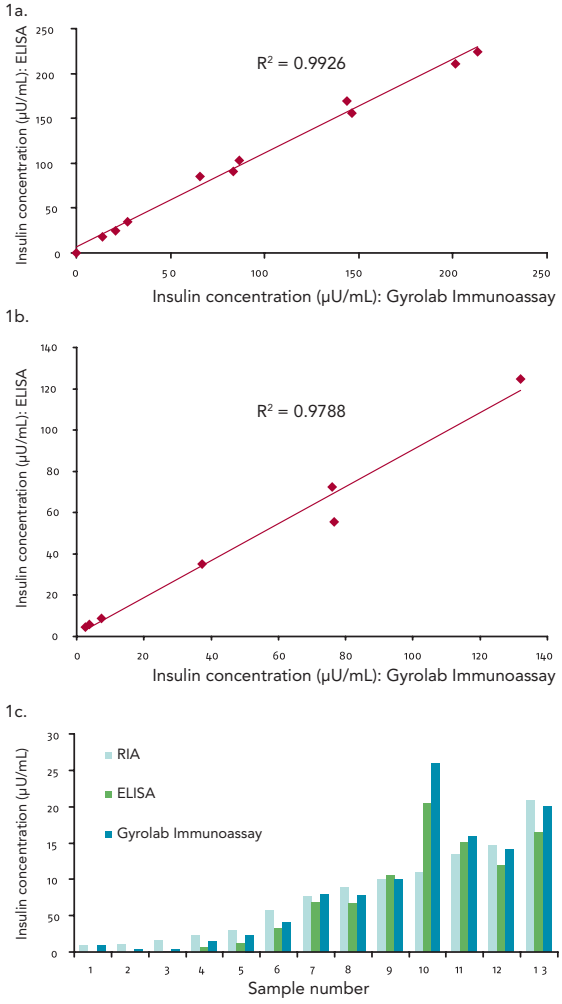


Figure 1 Correlation between Gyrolab immunoassay and ELISA when quantifying mouse insulin (a) and rat insulin (b) (both in Krebs-Ringer bicarbonate buffer). (c) shows the correlation between Gyrolab immunoassay, ELISA, and RIA when quantifying human insulin in serum. All data from the Diabetes Center Karolinska collaborative project.

Increasing throughput and reducing 'hands-on' time

The comparison in Figure 2 shows that the assay hands-on time was reduced to under 30 minutes (112 data points) using the automated Gyrolab® immunoassay from over 1 hour for the ELISA (96 data points). The overall assay time was reduced to just over 1 hour (Gyrolab platform) from 2.5 hours (ELISA).

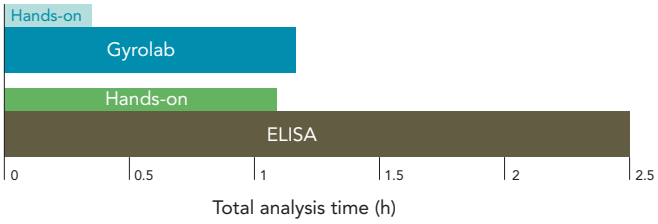


Figure 2 'Hands-on' time and total analysis time for Gyrolab and ELISA (Karolinska Institutet), generating 112 and 96 data points respectively.

Novo Nordisk

The research group at Novo Nordisk focuses on pre-clinical development and research around metabolic processes. Within the diabetes therapy area they have developed an insulin analogue, insulin-aspart[3] (insulin-Asp), and a two-sided ELISA for quantification of this analogue (Andersen et al. 2000). This assay is time consuming and uses large sample volumes. For the collaboration with Gyros Protein Technologies, the existing Novo Nordisk validated antibody pair could be directly transferred to Gyrolab immunoassay platform, due to the system's open, flexible format.

Excellent spike recovery

Figure 3 presents recovery data from plasma, serum, and buffer spiked with insulin-Asp. These results indicate that there were no significant matrix effects when using Gyrolab platform.

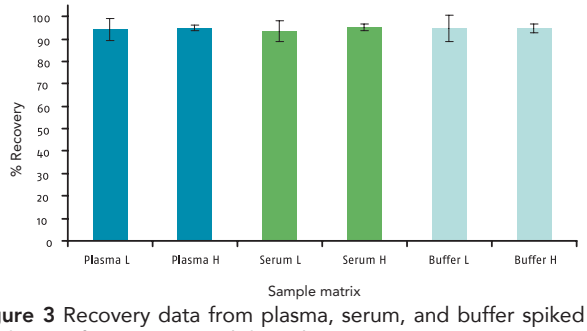


Figure 3 Recovery data from plasma, serum, and buffer spiked with insulin-Asp from Novo Nordisk study. L = 3.6 µU/mL, H = 115 µU/ mL. Average of five replicates.

[1] Insulin is an essential hormone that is released from beta cells in the pancreas. It regulates sugar production in the liver and the uptake of blood sugar and glucose in the muscles and fatty tissues.

[2] Mercodia, Uppsala, Sweden; 10–1149 (mouse), 10–1124 (rat).

[3] Proline at position 28 of the B chain of human insulin is replaced by aspartic acid generating insulin-aspart: a more rapidly acting insulin (only 10–15 mins), which can, therefore, be administered closer to meal times.

More samples analyzed per run

Up to 112 data points can be generated from each Gyrolab Bioaffy™ CD. In addition, up to five CDs can be processed in the same run for a total of 560 data points per 5 CD run. The standard curves for insulin-Asp, run on five CDs in one run, show excellent inter-CD reproducibility (see Figure 4). This means that standard curves only need to be generated once, per five CDs, leaving a larger number of data points available for samples per CD.

The results generated by Gyrolab immunoassays correlate well with the established techniques, with the additional advantage of using smaller sample volumes. See Table 1 for a comparison of sample volumes used in each of the techniques. Gyrolab immunoassay is able to generate a great deal of information from small sample volumes. For example, in one sample, 2 replicates analyzing analyte x and 2 replicates analyzing analyte y require no more than 4.5 µL of sample.

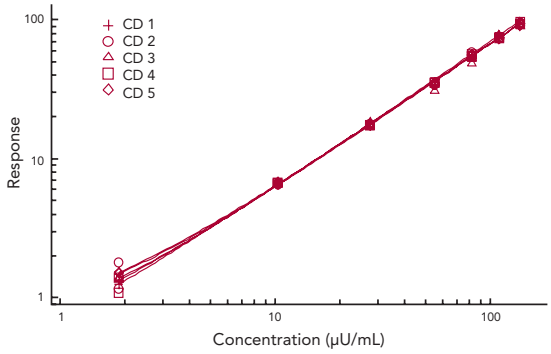


Figure 4 Standard curves for insulin-Asp from Novo Nordisk, each concentration run on five Gyrolab Bioaffy CDs in one run.

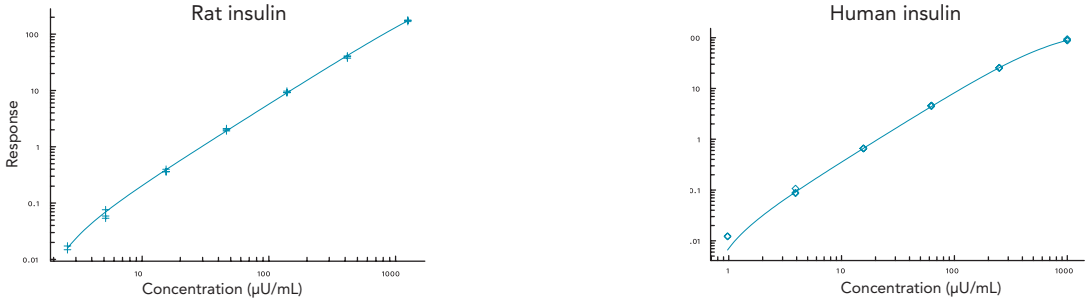


Figure 5 Standard curves from rat and human insulin. Diabetes Center Karolinska. 1 µU/mL ≈ 35 pg/mL ≈ 6 pmol/l. (Vølund et al. 1991)

Table 1 Comparison of the RIA and ELISA (Karolinska Institutet), ELISA (Novo Nordisk) and Gyrolab immunoassay techniques used in the two studies.

		RIA* (Karolinska Institutet)	**ELISA**** (Karolinska Institutet)	**ELISA*** (Novo Nordisk)	**Gyrolab immunoassay****
Sample volume	**1 replicate**	50 µL	25 µL	50 µL	3 µL
	2 replicates	100 µL	50 µL	100 µL	3.5 µL
	4 replicates	200 µL	100 µL	200 µL	4.5 µL
Measurement range	**human samples**	3.9–250 µU/mL	3–200 µU/mL	–	1–1000 µU/mL (KI)
	rat/mouse samples	3.9–250 µU/mL	3.7–137 µU/mL	–	1–1250 µU/mL (KI)
	insulin-Asp	–	–	1.9–144 µU/mL	1.9–144 µU/mL (NN)

* in-house developed technique
** commercially available technique

Results

Broad measurement range

The standard curves for rat and human insulin in Figure 5 show a linear range of more than three orders of magnitude. A broad measurement range allows the quantification of higher concentration samples without the need for sample dilution. For comparative data on measurement ranges for the techniques used in these studies, see Table 1.

The correlation between Gyrolab immunoassays and ELISA, when quantifying mouse and rat insulin, is shown in Figures 1a and 1b. Figure 1c shows the correlation between Gyrolab immunoassays, ELISA, and RIA when quantifying human insulin in serum.

Conclusions

Both of these collaborative studies show that Gyrolab platform is an effective tool for the development of high throughput insulin quantification analysis. In addition to matching the current ELISA and RIA technologies used, in terms of accuracy and reproducibility, Gyrolab platform also offers the following benefits:

- Smaller sample volumes can be used, essential when working with small animal models
- Increased throughput and precision from an automated and easy-to-use system
- Broad measurement range reduces need for sample dilution
- Excellent reproducibility, enabling more samples to be processed and more data points to be generated per assay run
- Cost reduction per data point as a result of reduced labor input and reduced reagent consumption

References

Grill V., Pigon J., Hartling SG., Binder C., and Efendic S. 1990. Metabolism. Vol. 39, No. 3. 251–258.

Volund A., Brange J., Drejer K., Jensen I., Markussen J., Ribel U., Sorensen A., and Schlichtkrull J. 1991. Diabetic Medicine. Vol 8, No. 9. 839–47.

Andersen L., Jorgensen PN., Jensen LB., and Walsh D. 2000. Clinical Biochemistry. Vol. 33, No. 8. 627–633.

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