Development of a High Sensitivity Assay for Mouse Plasma Insulin Using Single Molecule Counting

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1 BACKGROUND

Improvements in sensitivity, accuracy and precision of immunoassays are required to robustly quantify insulin levels in small volumes (5 µl) of mouse plasma, especially in studies using fasted animals.

OBJECTIVE

We report the validation of a highly sensitive immunoassay for mouse insulin with a low sample volume based upon the single molecule counting technology (Erenna™ System).

2 MATERIALS AND METHODS

Analytical Performance: Accuracy, linearity and lower limit of quantitation (LLQ) was assessed using pooled mouse plasma depleted of insulin by charcoal and then spiked with insulin at different concentrations. Within and between assay precision was assessed using plasma from fasting female mice, or control material.

Insulin Stability: Insulin concentration was measured with the Erenna method on plasma pools exposed to different storage conditions. The pool was either immediately frozen, stored on ice or at RT for 2 hr and then frozen at -20 or -80°C. Plasma insulin concentration was also measured after repetitive freeze-thaw cycles.

Intraperitoneal Glucose Tolerance: Mice were fasted for 16 hr and 50% dextrose (2g kg^{-1} body weight for males, 3g kg^{-1} body weight for females) was injected intraperitoneally. Either whole blood or heparinized plasma was collected from the tail vein at 0, 15, 30, 60, and 120 min post-injection. Glucose and insulin concentrations were measured. Glucose concentrations were determined using the Accu-Chek II glucometer (Roche Diagnostics). Plasma was separated by centrifugation, and stored at -80°C. Insulin was quantified using the Erenna Immunoassay System (Singulex, Inc.; Alameda, CA; www.singulex.com).

3 RESULTS: Analytical Performance - Stability

Figure 1: Erenna and Alpco Accuracy and Precision

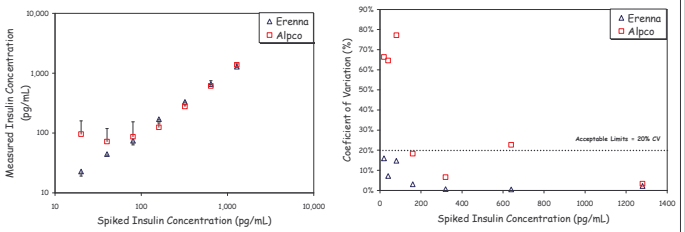

Table 1: Erenna and Alpco Analytical Performance

Performance Parameter	Erenna	Alpco
LLQ	20	125
Linearity	20-1,280	278-1,280
Precision at LLQ	16%	66%
Precision in fasting female mice	2-4%	5-26%

Table 2: Insulin stability under different conditions

Storage Condition	% Difference from Basal	
	-20°C	-80°C
2 hours @ RT, freeze	-8.1	0.4
2 hours on ice, freeze	-2.0	3.4
Number of Freeze/Thaw	% Difference from Basal	
1	2.3	
2	1.5	
3	4.6	

4 RESULTS: Intraperitoneal Glucose Tolerance (IPGTT)

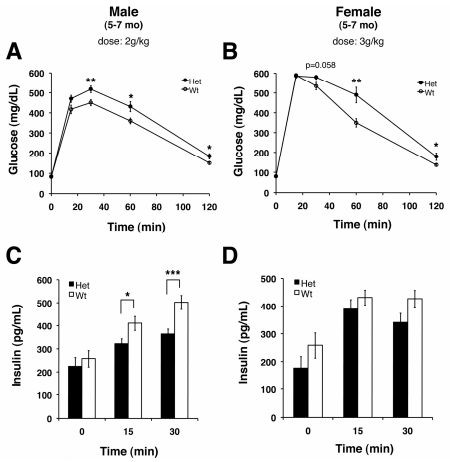

Figure 2: IPGTT

A and B:
IPGTTs in male and female Nampt +/- (closed circles) compared to controls (open circles). (A) Nampt +/- males (n = 15) and control males (n = 15). (B) Nampt +/- females (n = 15) and control females (n = 13).

C and D:
Plasma insulin concentrations (Erenna) from animals on A and B. (C) Nampt +/- males (n = 15) and control males (n = 15). (D) Nampt +/- females (n = 12) and control females (n = 15).
Nampt = Nicotinamide phosphoribosyltransferase (a.k.a. PBEF and visfatin)

All results are expressed as mean ± SE. $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$.

5 CONCLUSIONS

Using the Erenna System the following has been demonstrated:

- Insulin concentration can be measured with higher accuracy, lower limit of quantitation, linearity, and precision than reference method (Alpco).
- No changes in insulin concentration within the precision of the method under different storage conditions and freeze/thaw cycles were observed.
- Insulin concentration in fasted mice and IPGTT can be easily measured due to the increased sensitivity of the Erenna methodology.

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