Continuous Glucose Telemetry Calibration Guidance

The HD-XG and M series Glucose Implants are powerful tools for the collection of continuous glucose, temperature, and activity in mice, rats, and large animals. Accurate calibration requires that the user understand the following and perform calibration with diligence.

- **There are minimum calibration requirements over time**
  - A multipoint calibration should be performed within 5 to 14 days after implantation. This will establish a linear regression for calibration.
  - Single point calibration references should be collected at least once and preferably twice per week. This enables interpolation of the calibration should sensitivity loss occur.
  - A final multipoint calibration at the end of the study may be beneficial.
- **Do not calibrate during surgical recovery**
  - The electrical environment within the animal is transient for 4 to 7 days following surgery. During this period calibration should not be performed.
- **Use an accurate calibration reference**
  - The accuracy and precision of the assay which the continuous glucose telemetry implant is calibrated against is critical.
- **Consider the quality of your blood sample**
  - Blood samples should be obtained in a manner which avoids contacting skin, fur, gloves, or anything beyond what is needed for the assay.
- **Samples should be representative of blood where the glucose sensor is located**
  - In the ideal case blood should be samples from the same area of the vasculature where the glucose sensor is located. For many this will be impractical and sampling from the peripheral vasculature will be performed. In such cases care should be taken to minimize vasoconstriction or its effects.
  - Note: In rodents many researchers sample from the tail. At DSI this has been reliable for calibration in rats but not for mice. As such, in mice DSI recommends saphenous vein sampling for calibration.
- **Duplicate measurements are strongly recommended**
  - Collection of duplicate measures from the same blood sample allows for confirmation that they are in reasonable agreement and thus increases confidence in the reference. Further, if measures are not in agreement it provides an opportunity to recognize this and collect additional samples.
- **Timing of blood sampling is critical / Method of dosing also a consideration**
  - Blood samples should ideally be collected at a time when glucose has been relatively unchanged for at least 3 to 5 minutes. During a multipoint calibration this is not always possible and the timing of blood samples should be optimized

DSI ▪ 119 14th St. NW Suite 100 St. Paul, MN 55112
T:+1 (651) 481 7400 ▪ F:+1 (651) 481 7404 ▪ Toll free: 1 (800) 262 9687
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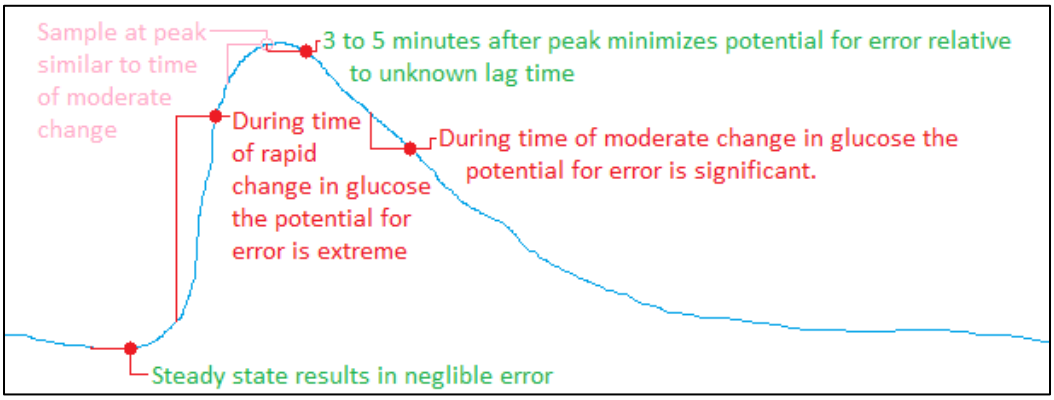
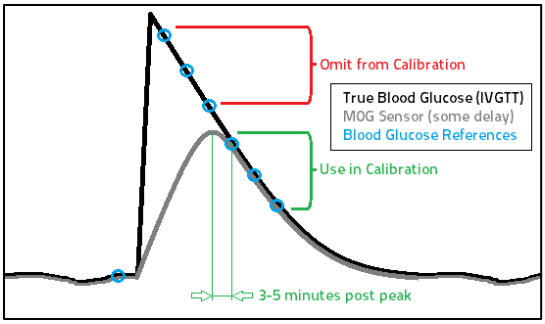
to minimize the impact of any possible time lag between the blood glucose in the central versus peripheral vasculature.



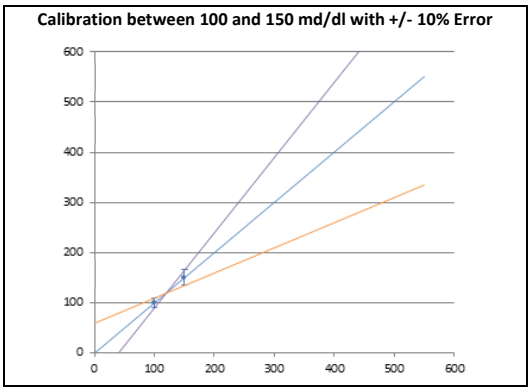
- Our recommended process for a multipoint calibration using a glucose or insulin tolerance test with oral or intraperitoneal dosing is to assay glucose prior to dosing, then to dose, then to observe the glucose signal, and then to assay glucose 3 to 5 minutes after the peak or nadir is observed.

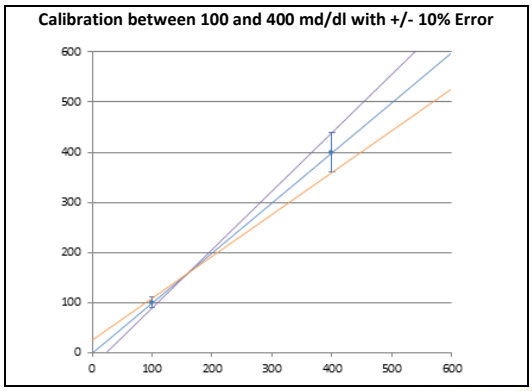


- If intravenous dosing was used for the GTT then two additional samples at approximately 10 and 15 minutes after the peak is observed are recommended.
- Note: calibration through means other than a GTT or ITT are possible provided low and high steady state glucose levels can be achieved.

□ **During multipoint calibration a wide range is beneficial**

- During a multipoint calibration it is recommended to have a change of at least 200 mg/dl (11.1 mmol/l) between the low and high references. This will both provide a more accurate calibration and minimize the impact or error within and outside that range as shown in the below plot examples.





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- For a GTT in a euglycemic animal this typically will result in a fasted glucose level of 70-90 mg/dl and post-peak glucose value of at least 290 mg/dl.
- Note: If the calibration low is measured below 60 mg/dl (3.3 mmol/l) it is recommended to also obtain an intermediate reference closer to 100 mg/dl (5.6 mmol/l) as the linearity of some glucose sensors deviates at very low values.

□ **Hypoglycemic range considerations**

- If a high level of accuracy at glucose levels below 60 mg/dl (3.3 mmol/l) is required special considerations are needed. Please contact DSI to discuss.

□ **Software Interaction for Calibration**

- The process for interacting with the software to enter or modify calibration references and to enable or disable the use of these references is contained in that software manual or user guide. It is recommended that you read and understand this section of the manual.

If you desire to understand these items further or discuss how best to implement in your application please contact DSI at glucose@datasci.com to schedule a technical discussion.

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