Has The Promise of non-invasive Glucose Testing Been Realized?
Progress and Hurdles

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Progress?

- **Several published optical methods**

- **Determination of glucose in aqueous solutions by NIR, Raman, Mid-IR, close to the physiological concentration range**

- **Tracking of changes in glucose concentration during a meal tolerance test, glucose clamps, and spot tests**

- **Limited clinical studies**

- **The powerful mathematical algorithms used and limited data sets cast shadow on the validity of some methods**

Hurdles/Limitations of non-invasive glucose testing

- Sensitivity
- Specificity
- Compartmentalization issues
- Body interface and physiological noise
- Multivariate calculations and error estimates

Sensitivity

- Very low molar absorbtivity of glucose
- No enzyme amplification as in the in-vitro methods
- No colored substrates with high molar absorptivity (NADH or dyes)
- Undefined properties of optical window (skin)

Specificity

- In vitro glucose determinations derive their specificity from the enzyme-substrate reaction

- NI glucose determinations derive their specificity from the use of multivariate calculations

- Require a training/calibration set to generate data from prediction data sets

- Overlap of calibration and prediction data sets And data over-fitting present major problems

Compartmentalization

- In vitro Glucose determinations use venous or capillary blood outside the body in defined reaction vessels
- NI glucose methods sample tissue volumes that include cells, interstitial fluid, and arterial and venous blood
- Skin properties and body physiological response to glucose may affect signal

Calculations and error limits

- In vitro glucose methods use single variable fit and calculate SD and CV that vary with glucose values
- NI methods use multivariate calculations that result in a constant standard error of prediction over the whole glucose range
- Even if all problems are solved NI will have a problem at low glucose (hypoglycemic) concentrations

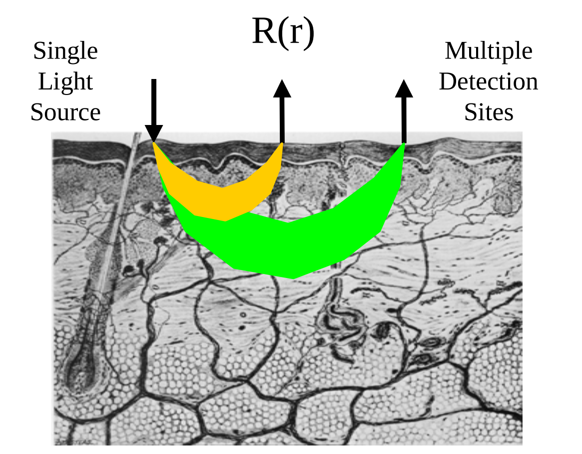
Will discuss two issues

1. Role of the skin

2. Error limits

3. What is needed

Localized reflectance of human skin

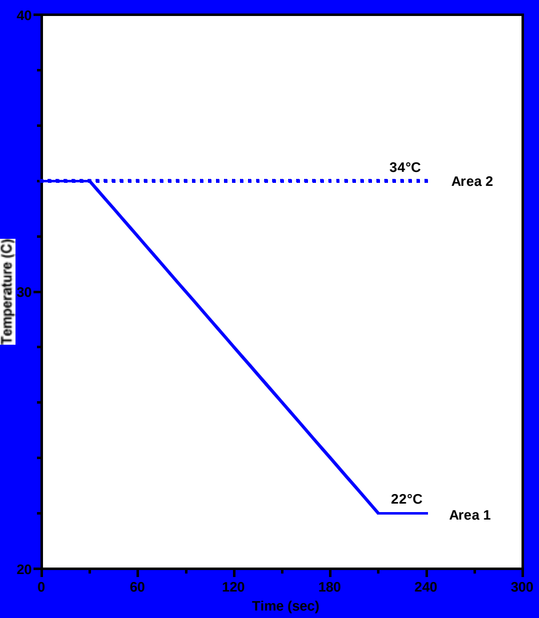


Infrared light passes through the skin and interacts with blood and tissue.

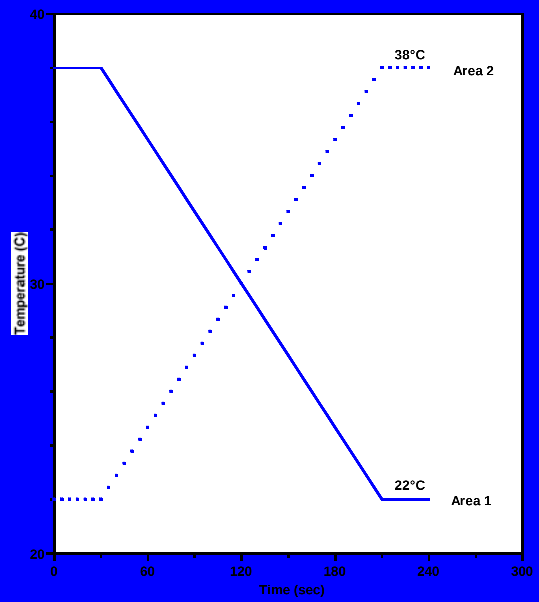
- Light is injected into the skin via a 400 micron fiber and collected at different distances
- Light intensity at each source-detector distance is determined
- Wavelengths 590 to 980 nm
- Temperature is controlled and varied via TE elements

Temperature controlled disc surrounding the optical fibers at center

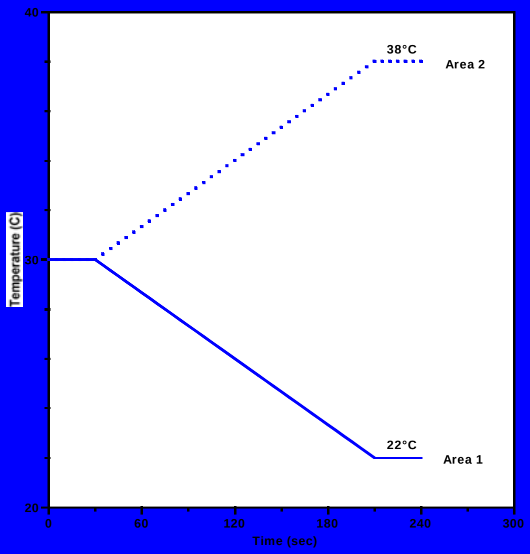
Temperature-modulated localized reflectance of human skin



Temp. Program 1



Temp. Program 2



Temp. Program 3

Yeh et al, J. Biomedical Optics 8 ;190-205 (2003)

A function f is derived from optical signals at two areas of the skin:

$$f(R_{A1T1}, R_{A1T2}, R_{A2T3}, R_{A2T4}) =$$

$$[\ln (R_{A1T1} / R_{A1T2})] - [\ln (R_{A2T3} / R_{A2T4})] \qquad (1)$$

R_{AiTj} is the localized reflectance within skin area i at time. f was determined at 4 sou

detector distances and at 4 wavelengths and used to generate a discriminant function

$$D = \sum_i \sum_j a_{ij} (\delta_i f_i)(\delta_j f_j) + \sum_i a_i \delta_i f_i + a_o \qquad (2)$$

where

$$\delta_i = 1 \; or \; 0; \quad \text{and} \sum_i \delta_i = K \qquad (3a)$$

$$\delta_j = 1 \; or \; 0; \quad \text{and} \sum_j \delta_j = K \qquad (3b)$$

a_{ij}, a_i, and a_0 are constants, and i or j are indices to specific combinations of wavelength and source-detector distance. K limited the total number of wavelength/source-detector distance combinations used in D, and δ_i and δ_j were determined from the training set through a leave-one-out cross validation procedure.

A subject is classified as diabetic if $D>0$, and non-diabetic if $D<0$.

Prediction of the diabetic status at 120 seconds after probe-skin contact

Temp. program	Optical Test Result	True Status	
		Diabetic	Non-diabetic
1	Diabetic	11	3
	Non-diabetic	1	9
2	Diabetic	11*	1*
	Non-diabetic	0*	9*
3	Diabetic	10	0
	Non-diabetic	2	12

*Total # of data points is 12, 3 points were rejected as they resulted in outlying signals.

Biological noise affecting skin optical response

- 15 Insulin dependent diabetic patients,
- In-hospital stay for 3 days, two to three at a time
- Reference method, capillary blood glucose
- Known caloric intake
- Use medication as prescribed by their physicians
- Data of day one and two are used as a calibration set
- Data of day three as a prediction set
- Noise ceiling was estimated by using the calibration set to predict the third day data after randomizing the glucose values
- Calculated the R^2 and determined the when R^2 ranked higher than the R^2 for fitting random numbers

Biological Noise

Parameters	Years of diabetes duration	
	< 20	> 20
Number of volunteers	8	7
Mean age (years)	43.3 ±13.8	55 ± 8.2
Duration of diabetes (years)	11.1 ± 4.2	24.3 ± 7.3
Body mass index	30.4 ± 7.3	28.5 ± 2.9
%HbA1c	7.2 ± 1.1	6.8 ± 0.9
Number of females	6	0
Number of males	2	7
Number (%)with R^2 ranking above noise ceiling	6 (75%)	2 (29%)
Number (%)with R^2 ranking below noise ceiling	2 (25%)	5 (71%)

Lowery et al, J. Biomedical Optics. In press

Observations

- Skin of diabetics differs from the skin of non-diabetics Human skin is affected with diabetes
- Diabetes affects optical / thermal properties of human skin
- It is possible to track glucose concentration changes for some patients, using thermo-optical response of human skin
- Duration of diabetes, and or gender affects thermo-optical response of human skin and the correlation between these properties and blood glucose levels

Even if detection is achieved, there is a problem with error estimates

1. Calibration and prediction is patient-specific using multivariate analysis

2. Multivariate calculations result in SEP. standard error of prediction, over the whole glucose concentration range

3. In-vitro calculations using single variable gives a standard deviation at a given concentration.

4. Calculated SEP may present a problem at low hypoglycemic glucose concentrations

Simulation of error estimates

Assumptions:

1. Home glucose monitor data for a single patient run over a period of time will be used to calibrate NI measurements

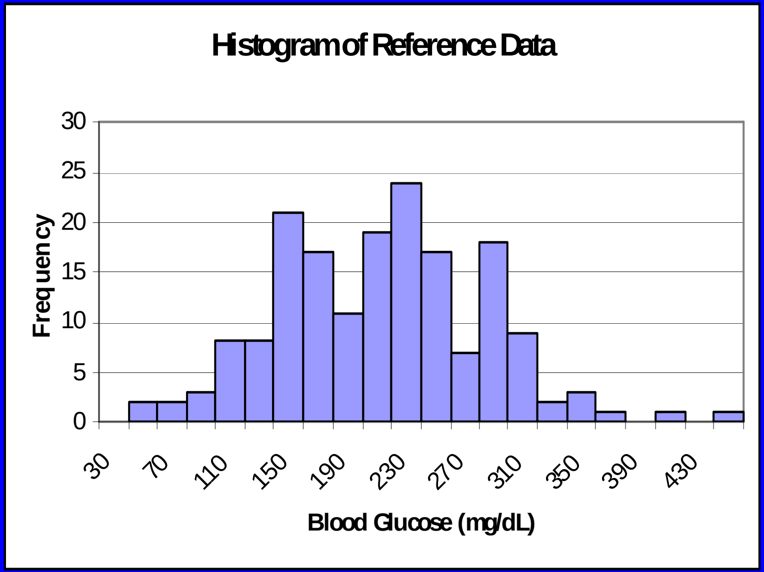
2. NI results will be less precise and less accurate than in-vitro results

3. Simulated imprecision by adding a constant error to each data point and calculating the SEP for new fit to the in-vitro data

4. Simulated intercept bias and slope bias between simulated in vivo data and in vitro data

Simulation Reference Data set

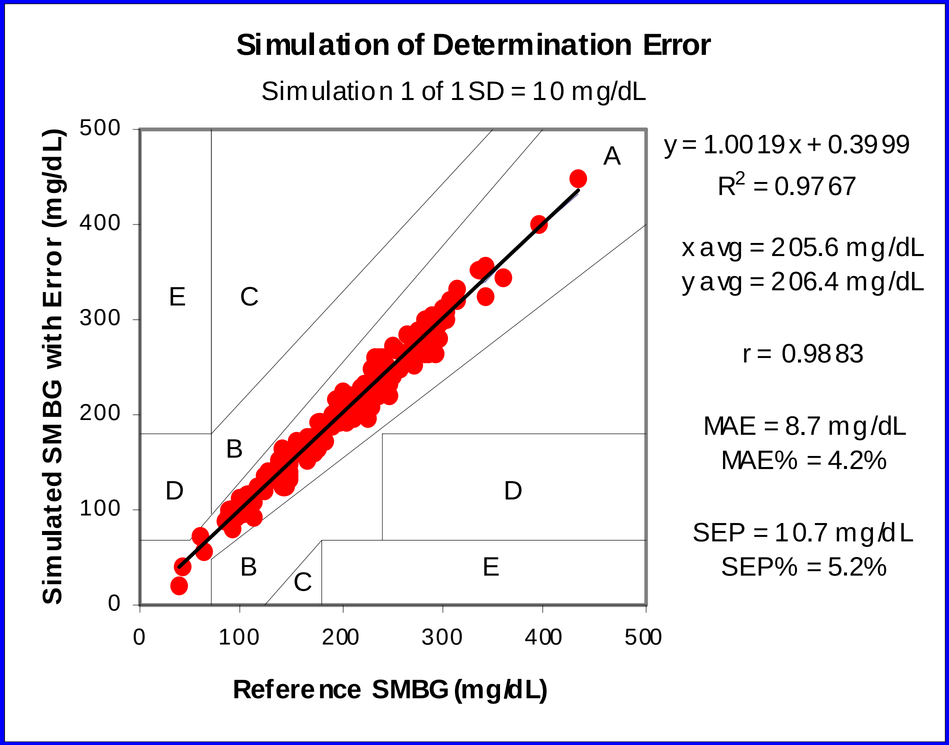
176 In-vitro data points provided by an insulin-dependent diabetic using his home-glucose meter

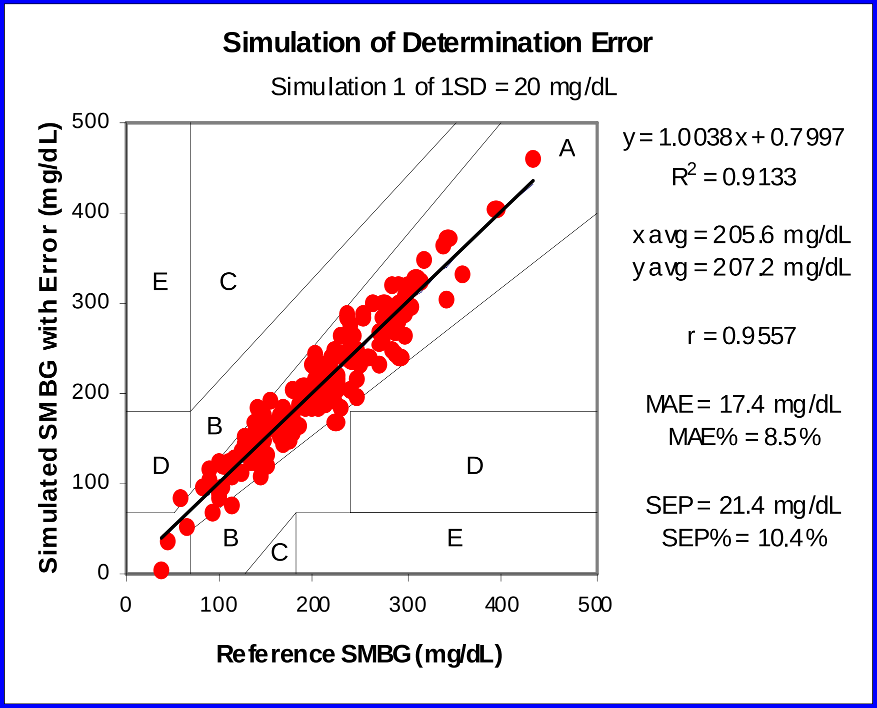


Prediction data: Derived from the reference data set by including imprecision, slope bias and intercept bias

Imprecision 1

A 10 mg/dL imprecision led to SEP = 10.7 mg/dL
A 20 mg/dL imprecision led to SEP= 21.4 mg/dL



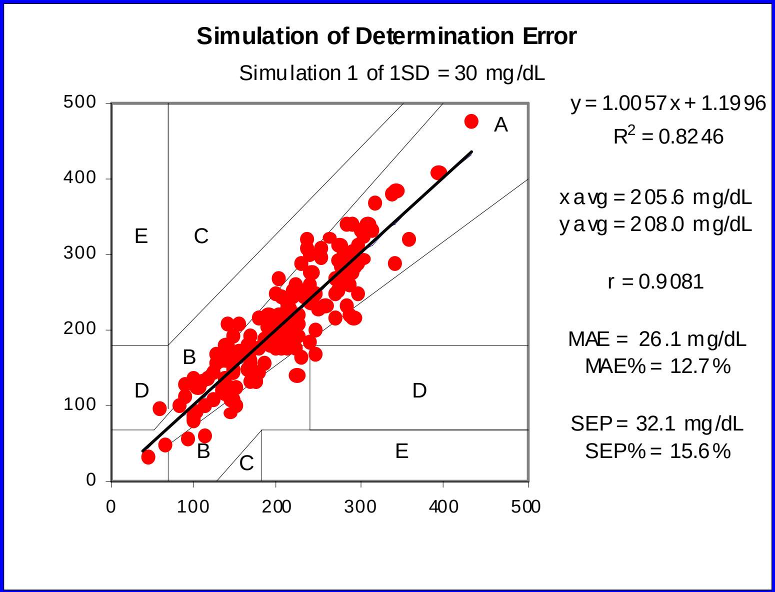


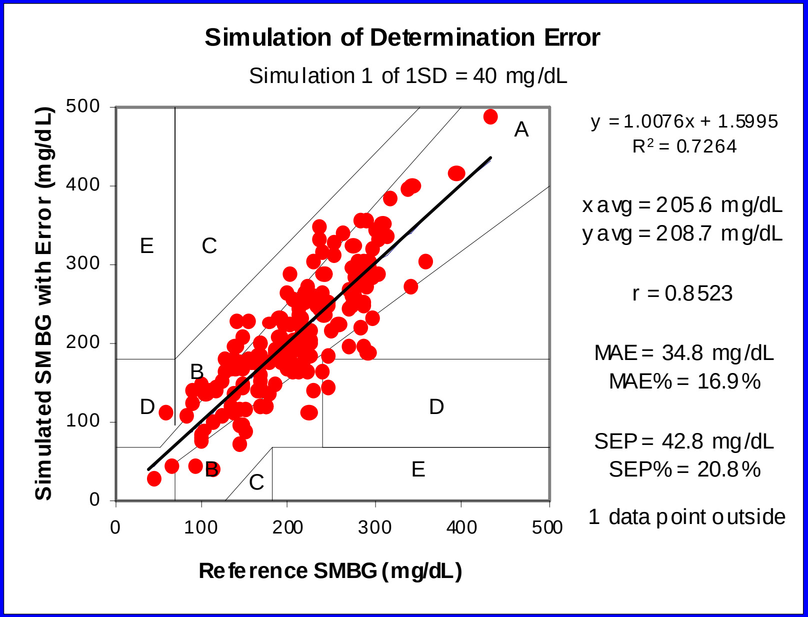
Khalil and Jeng, Clinical Chemistry, submitted for publication

Imprecision 2

A 30 mg/dL imprecision led to SEP = 32.1 mg/dL
A 40 mg/dL imprecision led to SEP= 42.8 mg/dL

Data points in erroneous zones on the Clarke Error Grid will lead to erroneous intervention and endanger patient's life





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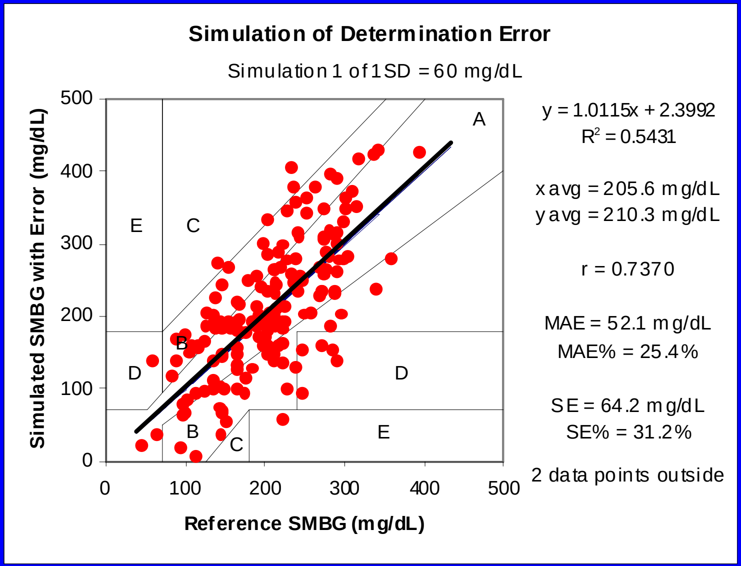
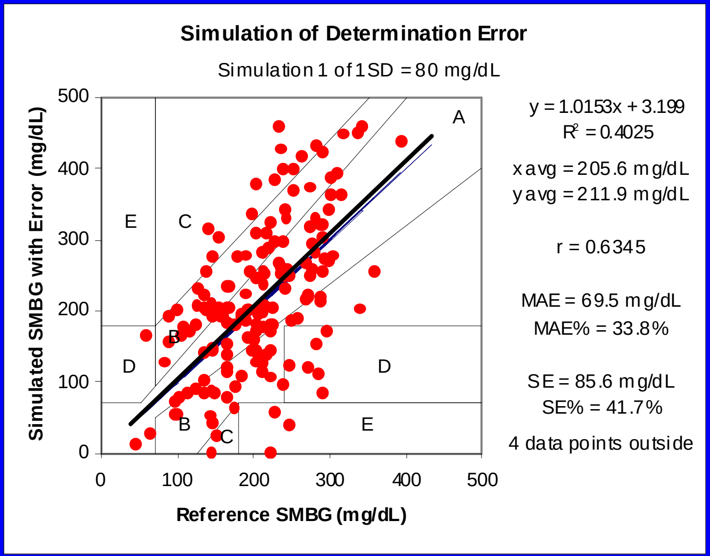
Imprecision 3

A 60 mg/dL imprecision led to SEP = 64.2 mg/dL
A 80 mg/dL imprecision led to SEP= 69.5.8 mg/dL

More data points in erroneous zones on the Clarke Error Grid will lead to erroneous intervention and seriously endanger patient's life

Approaches the case of a random distribution





Example data

- Arnold; Transmission through the tongue , 5 patients spot test, SEP > 54 mg/dL

- Heise, reflectance of oral mucosa, OGTT, SEP = 54 mg/dL

- Samann et al, 10 patients, leave-one-out cross validation
  - Short term SEP 18.5 mg/dL - 54 mg/dL
  - Long term experiment 55.8 to 6 108 mg/dl, one patient 646.2 mg/dL

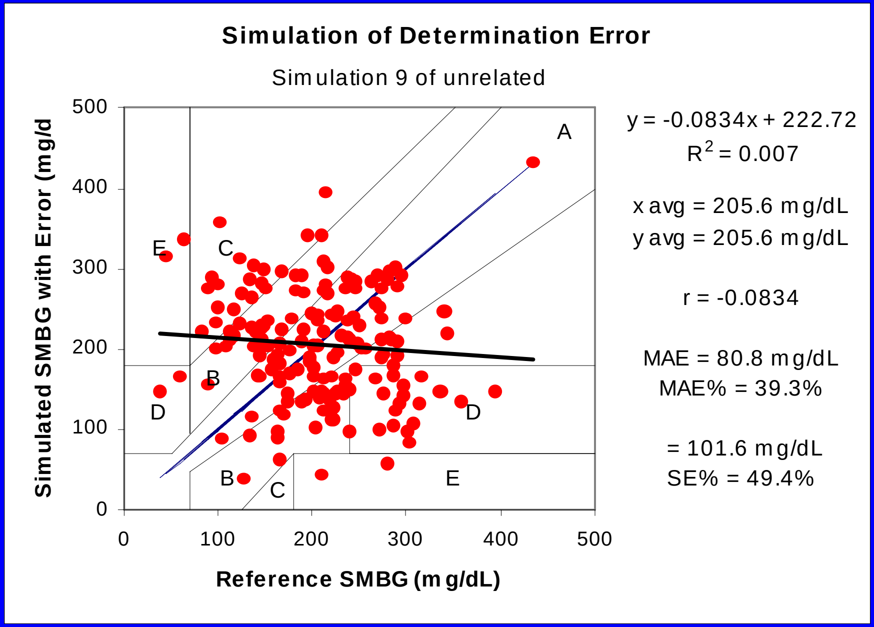
- Malin et al, selected population, 25 mg/dL

- For a review of latest performance: Khalil, Diabetes Technology and Therapeutics 5; 660-697 (2004)

Fitting to a Random Distribution

SEP of a random distribution is 80.8 mg/dl

Present NI methods are not equivalent to Random Distribution but still are far away from being quantitative



What do we need

1. Improve sensitivity of the measurements
   better selection of glucose-related signal
2. Improve specificity
   Body interface issues
   Body/skin physiology issues
   Calibration issue (sub-population calibration)
   Decrease number of variables
3. Compartmentalization:
   Define and target small sample of tissue where glucose distribution can be homogeneous
4. Error estimate
   Must be narrowed, even tighter than the in-vitro case, if multivariate analysis is used