

Clinical Evaluation of AGE Fluorescence for Noninvasive Screening for Type 2 Diabetes

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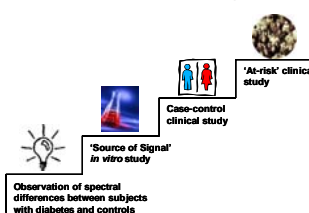
Introduction

In the US, the number of people estimated to have undiagnosed type 2 diabetes grew by over 20% between 2002 and 2005. In addition, the Diabetes Prevention Program has shown that certain interventions are effective at preventing or delaying the onset of diabetes in individuals with impaired glucose tolerance. VeraLight is developing Scout, a noninvasive instrument for detection of pre-diabetes and diabetes to address the need for a better screening tool.

The Scout development was spurred by an observation that the skin of individuals with diabetes is spectrally different than individuals without diabetes. An *in vitro* study (2003) implicated skin advanced glycation end-products (AGEs) as a likely source-of-signal. As a result, VeraLight tested a noninvasive pre-diabetes and diabetes detection method based on fluorescence spectroscopy in a case-control population (2005). The noninvasive method does not require fasting.

The most recent clinical study (2006) of detection of abnormal glucose tolerance is the culmination of four years of work and is a head-to-head evaluation of Scout and fasting plasma glucose (FPG) in a naïve, 'at-risk' population. The oral glucose tolerance (OGT) test was used to adjudicate truth. The results show that Scout outperforms FPG as a screening tool for pre-diabetes and diabetes.

Scout Development Progression



Current Research Prototype



Future Clinical Prototype



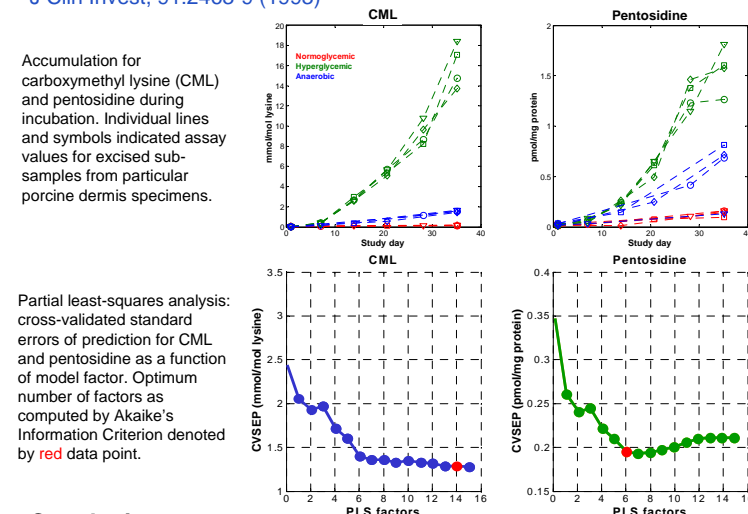
In Vitro Source-of-Signal Study

Experimental design

- Freshly-harvested porcine dermis
- Specimens subdivided and placed into 1 of 3 media: Normoglycemic, hyperglycemic or hyperglycemic/anaerobic
- 5 week duration

AGE assay method

- Technique of Dyer et al, Accumulation of Maillard reaction products in skin collagen in diabetes and aging, J Clin Invest, 91:2463-9 (1993)



Conclusions

- Spectral changes strongly correlated to AGE progression
- Active spectral regions consistent with collagen cross-linking
- Next steps include confirmation of *in vivo* source-of-signal

Case-Control Clinical Study

Experimental design

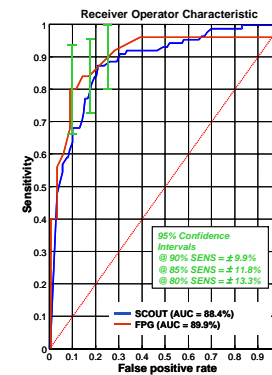
- 72 subjects
 - 36 with type 2 diabetes
 - 36 not previously diagnosed with DM
- Ages ranged from 30-76 years
- Two separate visits: fasting and random
- Capillary blood glucose and noninvasive measurements performed during each visit

Noninvasive disease classification

Discriminant analyses were applied to the fluorescence data to assess disease classification performance in subject-out cross-validation for the entire cohort

Conclusions

- Noninvasive classification in case-control was comparable to that of FPG



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Are Low Molecular Weight Serum AGEs the Source-of-Signal?

Motivation

- Test premise that serum AGEs diffuse into the interstitial space of the dermis and correlate with the Scout noninvasive measurement

Experimental design

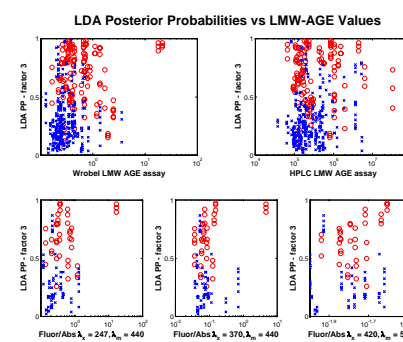
- 160 participants in the 'at-risk' clinical trial donated serum samples for LMW-AGE assay
- Mixture of subjects with type 2 diabetes (self-declared) and naïve, 'at-risk' subjects
- Each serum sample subdivided and processed in 3 assays:
 - Method of Wrobel et al., Clin Chem, 43(9), 1563-9 (1997)
 - Method of Thomas et al., Kidney Int, 66, 1167-1172 (2004)
 - Bulk fluorescence, Baynes
- 3 noninvasive measurements per subject

Spectral analysis

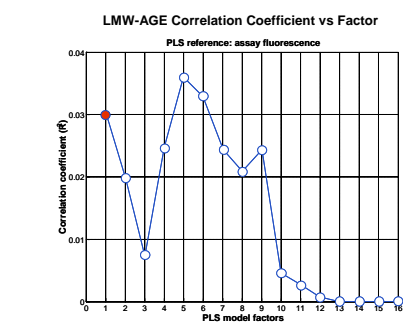
- Subject spectra characterized by posterior probabilities from linear discriminant analysis (LDA) in subject-out cross-validation
- LDA factor selected by optimal spectral disease class separation
- Quantitative, partial least-squares (PLS) models built using assay reference values

Conclusions

- Qualitative inspection reveals negligible correlation between spectral scores and biochemical assay results
- Quantitative analysis confirms absence of relationship between LMW-AGE assay values and skin fluorescence spectra



Comparison of LMW AGE results and spectral posterior probabilities from LDA analysis. Red symbols (o) denote results from subjects with type 2 diabetes while blue data points (o) represent values from control subjects without disease. Subjects received 3 optical measurements.



Correlation results from partial least squares analysis of subject spectra applying bulk fluorescence assay results as reference values.

Clinical Study on 'At-Risk' Population

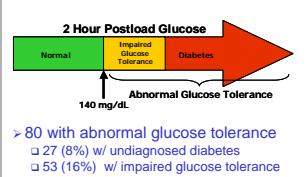
Experimental Design

- 328 naïve subjects 'at-risk' for type 2 diabetes or pre-diabetes
 - Risk factors included age, first degree relative with type 2 diabetes, hypertension, dyslipidemia, body mass index > 25, ethnicity, previous gestational diabetes, baby weighing more than 9 lbs at birth and/or polycystic ovary syndrome
 - Most subjects had two or more risk factors
- Subjects had to fast overnight (> 8 hours) before each morning appointment
- At first appointment obtained informed consent, patient history plus measured fasting plasma glucose and skin fluorescence
- At second appointment measured oral glucose tolerance and skin fluorescence
- Oral glucose tolerance test used to adjudicate truth

'At-Risk' Cohort Demographics

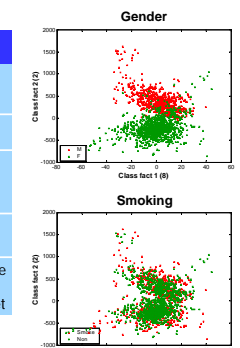
Age	Gender	Ethnicity
21-30	Male	Caucasian
31-40	Female	Hispanic
41-50	Total	African Am
51-60		Native Am
61-70	Smokers	Asian
71-80	Yes	East Indian
81+	No	Other
Total	Total	Total

Disease Classification

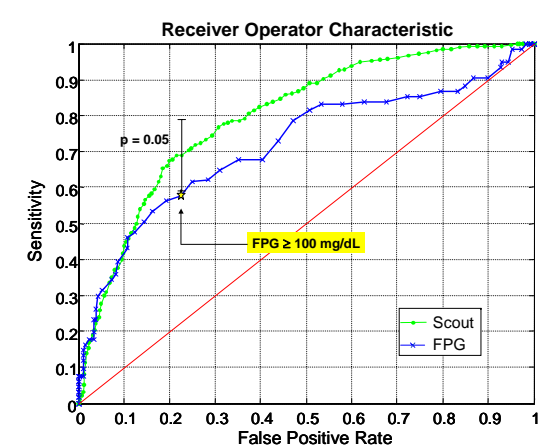


Examination of Potential Confounding Factors

Factor	Issue	Method	Conclusion
Subject Age	Natural increase in skin fluorescence as subjects age	Use subject's age to remove trend	No impact
Gender	Physiological skin differences	Optical sorting	No impact
Skin color	Melanin content impacts signal intensity and optical pathlength	Intrinsic fluorescence correction	No impact
Smoking	Smoking may corrupt skin fluorescence signal	none	No impact
Diet	Ingested AGEs may corrupt skin fluorescence signal	none	Good performance despite not controlling for diet



Abnormal Glucose Tolerance Detection



Test Method	SENS (%)	SPEC (%)	AUC (%)
Scout	68.8	77.4	79.8
FPG ≥ 100 mg/dL	57.5	77.4	71.5
Absolute Δ	11.3	-	8.3
Relative Δ	19.7	-	11.6

Clinical Study Summary

- Scout significantly outperforms fasting plasma glucose as a screening test
- Scout's lack of a fasting requirement, overall convenience and superior accuracy will facilitate opportunistic screening at the point-of-service