

Final report HRC 09/662: Is measurement of skin autofluorescence an effective method for both screening and monitoring of diabetes?

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Background

There is considerable evidence that measuring the levels of advanced glycation endproducts (AGEs) in skin provides an index of integrated glycaemic exposure (1,2). Levels of AGEs in skin may be determined by biopsies or non-invasively by measuring skin autofluorescence (SAF). Studies have shown SAF intensity is approximately two-fold higher in patients with diabetes than in control subjects (3-5). To date, technology using this difference in SAF as a means to screen for diabetes has not achieved sufficient sensitivity to discriminate between diabetic and non-diabetic people. Instead, SAF devices have been limited to assessing vascular risk associated with increased AGE levels in tissue (6). Recent developments in SAF technology, initiated at Canterbury University, have improved the sensitivity of the method by incorporating an enhancement step using shorter wave ultraviolet (UV) light in the scan sequence. This technique is called enhanced SAF (ESAF). The ESAF method was validated and assessed by a series of pilot investigations (Prototype 1) carried out between July 2009 and March 2010, using funds obtained from other grant applications.

The primary aim of the current study was to further develop the ESAF method to provide a sensitive index for non-invasive screening of diabetes. The secondary aim of the study was to confirm the relationship between ESAF measurements and the presence of diabetic vascular complications.

ESAF methodology

The ESAF device illuminates the flexor surface of the forearm using a 360nm light source. Emission and excitation light from the skin are measured approximately 20 times between 355-700 nm. The skin is then exposed to a short period of 254nm UV and the scans repeated. Enhancement of the fluorescent signal in the 450-500nm region of the spectrum is then calculated and expressed as derived units.

On the basis of the results of the pilot study (Appendices 1-3), a more sensitive fluorospectrophotometer was incorporated into the ESAF measuring device in August 2009 (Prototype 2), with the aim of increasing the signal to noise ratio of the measurements. Clinical evaluation of this prototype between September and October 2009 showed considerable variation in the scan data as a result of background interference from cutaneous vasomotion. New scan sequences were developed to standardise for this effect of vasomotion. During the early period of HRC funding, starting 1 March 2010, four different prototypes (3-6) of the ESAF device were evaluated. The results of these investigations led to further refinement of the scan sequence (Prototype 7) and incorporation of simultaneous skin colour measurement in Prototype 8. Clinical assessment of these two prototypes was carried out between April 2010 and February 2011, with Prototype 8 being used for a comparative study with an oral glucose tolerance test (OGTT). Table 1 summarises the timeline and main features of these prototypes.

Table 1. Timeline of ESAF development

Prototype #	Date	Scan 1	254nm UV	Scan 2
1 Pilot study	Jan 2009	30 secs	10-20 secs	30 secs
2 New fluorospectrophotometer	Sept 2009	30 secs	5-7 secs	30 secs
3 Effects of vasomotion incorporated In analysis	Nov 2009	120 secs	5-7 secs	120 secs
4 Doubled 254nm UV exposure	Feb 2010	120 secs	10-15 secs	120 secs
5 ¼ 254nm UV intensity with longer scan	Early Mar 2010	120 secs	60 secs	120 secs
6 ¼ 254nm UV intensity - determine optimum scan profile to measure time-related enhancement in ESAF.	Late Mar 2010	60 secs	30 secs	Sequence repeated x6
7 Increased exposure to ¼ 254nm UV and definitive scan sequence established	Apr-Sept 2010	60 secs	60 secs	Sequence repeated x3
8 Simultaneous measurement of skin colour.	Oct 2010- Feb 2011	60 secs	60 secs	Sequence repeated x3

Study participants

The study included five main groups.

1. Patients with diabetes mellitus, enrolled from clinical databases and advertisements in local newspapers.
2. Subjects with the metabolic syndrome, enrolled from clinical databases and advertisements in local newspapers.
3. Normal healthy control subjects, including staff members at the hospital and those enrolled from advertisements in local newspapers.
4. Subjects suspected as having diabetes mellitus undergoing an oral glucose tolerance test at MedLab South Laboratories, Christchurch.
5. Pregnant women screened for gestational diabetes by an oral glucose tolerance test at MedLab South Laboratories, Christchurch.

Assessment of all the ESAF prototypes involved the same basic profile of tests. Duplicate ESAF scans were carried on the flexor surface of the patient's forearm. The scan area was cleaned with a swab containing 70% isopropyl alcohol prior to the measurements. Skin colour was measured using a narrow-band reflectance spectrophotometer, with the data expressed as the erythema, melanin, R, G, B and CIE L, a, and b indices (7).

The following clinical parameters were also measured at the study visit: brief medical history including current medications, anthropometry (height, weight, waist circumference, BMI, and percentage body fat) smoking history (pack/years), non-fasting glucose, HBA_{1c}, mean HbA_{1c} in 1995-1999, 2000-2004 and 2005-2010, and plasma creatinine concentrations. Haematocrit and plasma bilirubin levels were also measured for prototypes 7 and 8.

Data analysis

Analysis of the scan data involved examining the spectra from 355-700 nm in approximately 8nm band widths, with the change in amplitude and shape of the curves in each bin being calculated. The data, standardised to readings at 360nm were expressed as the mean (\pm standard deviation) of the increase in amplitude in each bin following enhancement at 254nm UV. A linear fit was then applied to the spectra and the slope of the peaks analysed using Fourier transformation to obtain derived values of magnitude and offset (i.e. peak shape). The root mean square of the measurements was also calculated to determine signal noise caused by vasomotion or movement of the arm.

The scan and clinical data were then incorporated into a database for analysis of descriptive statistics and construction of ROC curves to assess the sensitivity and specificity of ESAF to discriminate between diabetes and non-diabetes. The relationship between the ESAF measurements and indices of glycaemic control (duration of diabetes and non-fasting glucose and HbA1c levels), and other clinical parameters (skin colour, haematocrit, plasma bilirubin and creatinine levels) were examined using correlation analyses.

The cross-sectional relationships between ESAF and indices of diabetic microvascular complications were examined by multiple linear regression analysis. Analysis of variance (ANOVA) was used to compare differences in variables between sub-groups of microvascular complications. Microvascular status was assessed by the presence or absence of 1) macular oedema, non-proliferative retinopathy or proliferative retinopathy, based on ophthalmic examination and retinal photography, 2) distal symmetric polyneuropathy or autonomic neuropathy, based on sensory tests and/or vibration perception thresholds measured by a biothesiometer, 3) nephropathy based on plasma creatinine concentration and urinary albumin concentration (normo- (<20 μ g/ml) micro- (20-200 μ g/ml) or macro (>200 μ g/ml)-albuminuria.

Results

Prototypes 2 and 3. Sept 1999 – Feb 2010

Prototype 2 incorporated a more sensitive fluorospectrophotometer and reduced exposure to 254nm UV. Data were collected from 88 individuals (41 females, 47 males; 61 non-diabetic, 24 type 2 diabetes and 3 type 1 diabetes). The study group included 5 Maori and 6 Asian subjects. Serial measurements were carried out in 28 subjects, with the majority having 3 measurements.

A high level of variability was observed in the scans. A total of 303 usable scans from the study population was obtained (101 scans from subjects with either diabetes or HbA1c levels \geq 43 mmol/mol and 202 scans from subjects with HbA1c levels < 43 mmol/mol). The study also assessed the effects of extraneous light on the scans and also whether or not UV blockers (sunscreens and moisturisers) affected the ESAF measurements. The clinical data of the patients is summarised in Table 2.

Table 2. Clinical data of subjects scanned with Prototype 2.

	Diabetes n=27	Controls n=61	P value
Gender (M/F)	16/11	31/30	NS #
Age (yr)	63.2 (13.6)	57.8 (10.9)	NS
BMI (kg/m ²)	30.0 (5.0)	30.1 (5.2)	NS
Waist circumference (cm)	104 (13)	102 (14)	NS
Body fat (%)	33.8 (12.0)	33.8 (12.0)	NS
Mean arterial pressure (mmHg)	103 (8)	102 (10)	NS
On antihypertensive agent (%)	64	37	0.03 #
Smoking history No/Ex/Current (%)	41/41/18	58/39/3	0.02 #
Pack years	14.1 (16.0)	6.8 (13.6)	0.03*
Duration of diabetes (yr)	9.5 (7.7)	-	-
Skin colour			
Erythema index	10.55 (1.85)	11.37 (2.28)	NS
Melanin index	34.23 (3.19)	34.75 (4.24)	NS
CIE L	38.12 (4.24)	37.48 (4.61)	NS
CIE a	14.20 (2.36)	14.77 (1.95)	NS
CIE b	11.34 (2.24)	12.19 (2.79)	NS
Non-fasting glucose (mmol/L)	9.1 (3.3)	5.5 (1.0)	<0.001*
HbA1C (mmol/mol)	58.5 (11.8)	39.0 (3.8)	<0.001*
Plasma creatinine (µmol/L)	86 (18)	84 (14)	NS

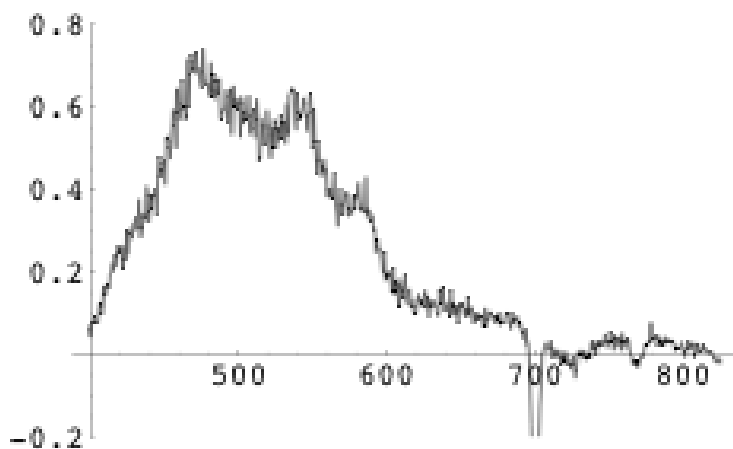
* Abnormally distributed data compared using Wilcoxin Rank Sum Test

Categorical variables compared using Chi-square test

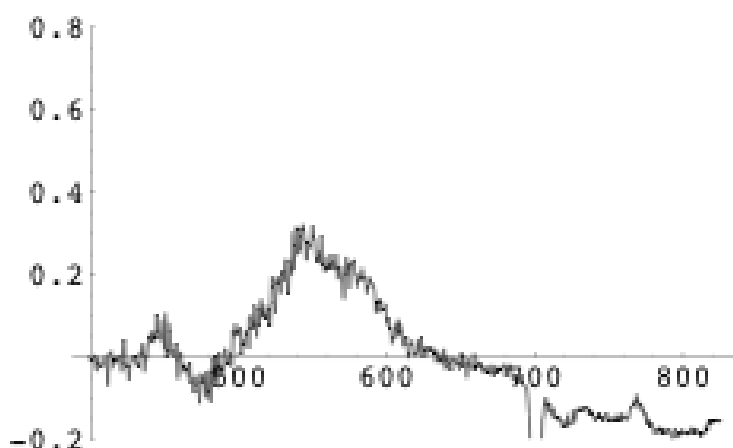
- The diabetes group had a higher prevalence of antihypertensive use, lower prevalence of smoking, and as expected, worse glycaemic parameters than the control group.
- Maximum light exclusion during the scans was achieved using a double-layer of black felt cloth fitted over the patient's arm.
- Application of moisturiser (SPF 15) and sunscreen (SPF 30+) both caused complete inhibition of the scans, with the machine "locking-up" and no data being obtained. Moisturiser without a UV blocker caused no difference in fluorescent intensity between pre- and post-application scans (ESAF without lotion $3.77 \pm \text{SEM } 1.37$ vs ESAF with moisturiser lotion $2.62 \pm \text{SEM } 0.72$; $p=0.38$). In order to avoid any interference from UV blockers, the patient's forearm was wiped thoroughly with a 70% isopropyl alcohol swab prior to scanning.
- Analysis of combined spectra of the diabetic and non-diabetic groups showed differences in fluorescent profile (Fig. 1). A distinct peak in the 450-500 nm region was observed in the diabetes group but not in the control group.

Figure 1. Spectra obtained with prototype 2 in the diabetes and control groups .

Average of 101 scans from patients with diabetes



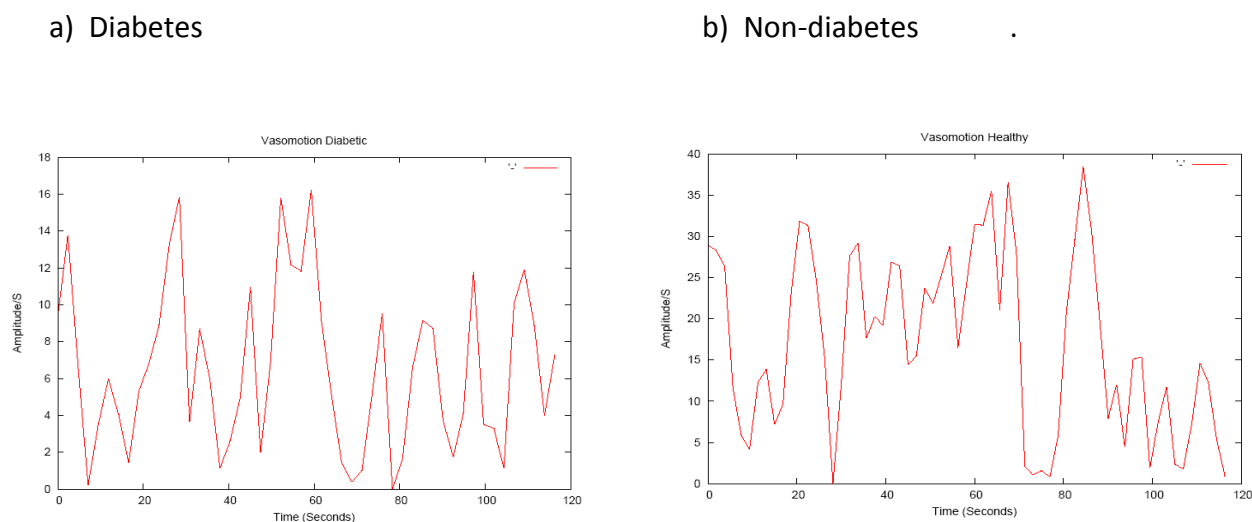
Average of 201 scans from healthy, non-diabetic controls



- A cyclical wave pattern with a frequency \cong 15-20secs was also observed in the spectra. These oscillations were considered to be a consequence of the spectrophotometer detecting changes in blood flow that corresponded to the 0.02-0.06 Hz wave pattern seen on Doppler scans that represent sympathetic-dependent vasomotion. These oscillations are an index of capillary bed perfusion (cutaneous vasomotion), which is controlled by contraction and dilatation of arterioles.
- The spectroscopic signal from cutaneous vasomotion was greater than the fluorescent signal from the glycated skin proteins and therefore resulted in large variability in the ESAF measurements. In order that protein fluorescence in the 450-500 nm region could be measured across several vasomotion waves, the duration of the scans was increased to 60 secs. It was also decided to double the 254nm UV exposure in an attempt to increase the amplitude of the fluorescent signal (Prototype 3).

- Examples of vasomotion in a diabetic and non-diabetic subject are shown in the following graphs (Figures 2a and 2b). The amplitude of the peaks in the non-diabetic subject were approximately two-fold higher than in the diabetic subject (note different scales for y axis in the two graphs).

Figure 2. Cutaneous vasomotion measured at 485 nm using Prototype 3.



Prototype 4. February 2010

- This prototype increased the exposure to 254 nm UV in order to increase the signal to noise ratio of the measurements. Exposure at 254 nm UV for 15 secs was found to yield maximum enhancement of SAF. To avoid erythema, subjects with fair skin (melanin index <32) were given 10 secs exposure, and those with darker skin (melanin index >32) were given 15 secs exposure.
- Analysis of scans indicated fluorescence of glycated proteins was also a time-dependent reaction, with heavily glycated proteins enhancing more rapidly. It was therefore decided to reduce the intensity of 254nm UV and increase exposure time. This was achieved by using a neutral density filter to reduce UV intensity four-fold and accordingly scan four times longer (i.e. 60 secs).

Prototypes 5 and 6. March 2010

- Analysis of the scans indicated the time-dependence of the photochemical reaction of glycated proteins in skin was considerably longer than initially thought. A small number of subjects (staff members) were given graded increases in exposure to $\frac{1}{4}$ intensity 254 nm UV for 80, 120 or 180 secs in order to assess whether this caused unacceptable erythema. As these longer exposure times were not associated with erythema it was decided to use a total exposure time to 180 secs in subsequent scans.
- Prototype 6 was introduced that involved 60 sec scans interspersed with 6 series of 30 sec exposure to $\frac{1}{4}$ intensity 254nm UV. After analysis of a small number of scans it was shown only three series of scans and enhancement steps were necessary to obtain usable data (Prototype 7).

Prototype 7. April – September 2010

This prototype was used during the period of HRC funding. The scan sequence is shown in the following figure.

Figure 3. Scan sequence of prototype 7.

Scan 1 60 secs n=20	254nm UV 60 secs	Scan 2 60 secs n=20	254nm UV 60 secs	Scan 3 60 secs n=20	254nm UV 60 secs	Scan 4 60 secs n=20
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Repeat scanning of the study cohort used for Prototypes 2 and 3 and also newly enrolled participants was carried out. Usable scans were obtained from 113 subjects (61 diabetics, 52 non-diabetics). Forty-five subjects had 4 scans over a 1-2 mth period to determine the reproducibility of the method. The clinical data of the subjects scanned using Prototype 7 is shown in Table 3.

Table 3. Clinical data of subjects scanned with Prototype 7.

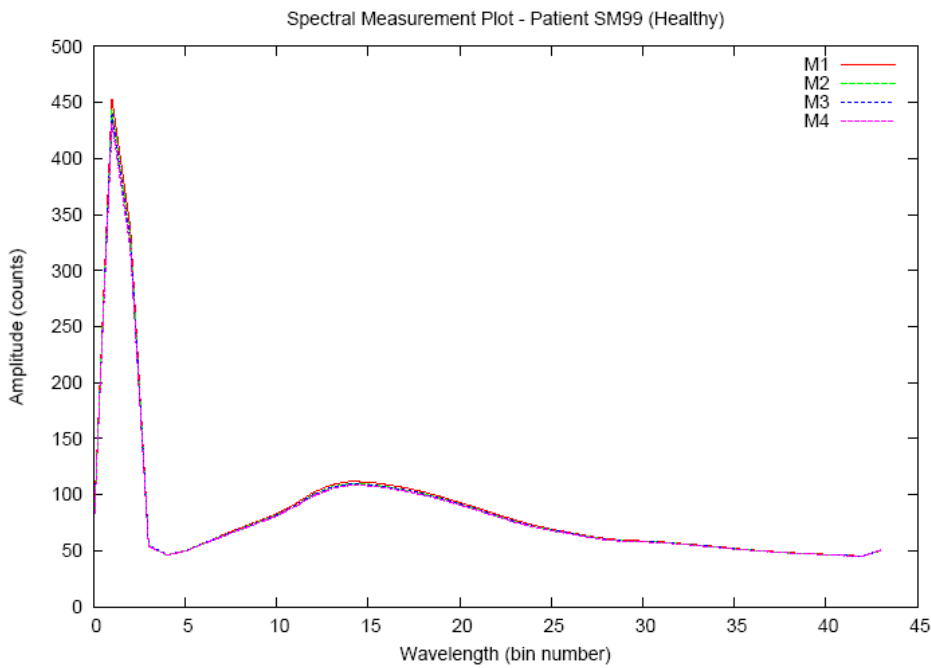
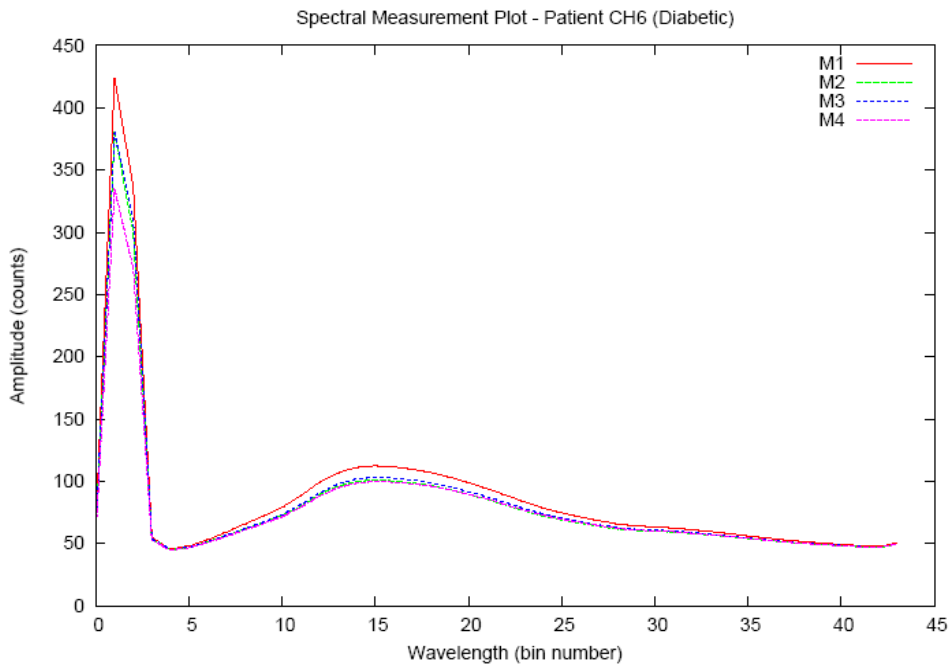
	Diabetes n=61	Control n=52	P value
Gender (M/F)	37/24	18/34	<0.01 #
Age (yr)	61.4 (11.2)	54.3 (10.3)	<0.001
BMI (kg/m ²)	31.2 (5.9)	31.7 (6.9)	NS
Waist circumference (cm)	104 (19)	102 (14)	NS
Mean arterial pressure (mmHg)	99 (8)	95 (12)	NS
On antihypertensive agent (%)	47	19	<0.001 #
Smoking history No/Ex/Current (%)	29/12/20	28/7/17	0.02 #
Pack years	13.3 (22.8)	11.0 (19.2)	NS*
Duration of diabetes (yr)	12.6 (8.8)	-	-
Skin colour			
Erythema index	14.8 (2.7)	15.6 (2.4)	NS
Melanin index	40.6 (5.8)	39.8 (5.3)	NS
CIE L	31.0 (5.0)	31.2 (4.6)	NS
CIE a	16.9(2.2)	17.9 (2.0)	NS
CIE b	13.3 (2.1)	14.5 (2.2)	<0.01
Non-fasting glucose (mmol/L)	10.7 (5.7)	5.6 (1.2)	<0.001*
HbA1C (mmol/mol)	64.5 (16.6)	39.9 (4.8)	<0.001*
Plasma creatinine (µmol/L)	95 (15)	81 (15)	<0.001

* Abnormally distributed data compared using Wilcoxin Rank Sum Test

Categorical variables compared using Chi-square test.

- The diabetes group contained a higher proportion of males, a higher prevalence of antihypertensive use, and as expected, worse glycaemic parameters than the control group.
- Representative scans from subjects with and without diabetes are shown in Figure 4. The diabetic subject has an increase in amplitude following the first enhancement step, whereas the non-diabetic subject does not.. This increase is apparent in bin numbers 10-25 that corresponds to a wavelength range of 435-555nm. The increase is greatest in bins 13-18 (460-500nm).

Figure 4. ESAF scan of diabetic and control subjects.
MI-4 corresponds to scans after enhancement steps 1-4, respectively.



- Mean ESAF arbitrary units measured at 467nm were significantly higher in subjects with diabetes compared to controls (Table 4).
- A correlation matrix between ESAF arbitrary units measured at 467nm and other clinical indices is shown in Table 5. A significant correlation ($p < 0.05$) was observed between ESAF and non-fasting glucose and mean HbA1c levels measured over the preceding five years. Interestingly, ESAF showed no significant correlation with HbA1c measured at the scanning visit. It was also noted that the degree of correlation between ESAF and mean HbA1c decreased over time.
- The erythema index also showed a significant correlation with ESAF, indicating that skin colour is a major determinant of ESAF. No correlation was observed between ESAF and cigarette smoking or renal function.

Table 4. Mean ESAF arbitrary units measured at 467nm using Prototype 7.

Index	Diabetic n=61	Control n=52	P value
Scan 1	0.0169 (0.0023)	0.0158 (0.0024)	0.007
Scan 2	0.0170 (0.0023)	0.0159 (0.0024)	0.011
Scan 3	0.0171 (0.0024)	0.0160 (0.0023)	0.012
Scan 4	0.0172 (0.0024)	0.0161 (0.0023)	0.010

Table 5. Correlation (Pearson's r) between ESAF at 467nm and clinical parameters.

	Scan 1	Scan 2	Scan 3	Scan 4
Non-fasting glucose	0.23 *	0.24 *	0.25 *	0.07
Last HbA1c	0.18	0.17	0.17	0.12
Mean HbA1c 2005-2010	0.29 *	0.27 *	0.27 *	0.26 *
Mean HbA1c 2000-2005	0.23	0.22	0.21	0.21
Mean HbA1c 1995-2000	0.20	0.18	0.18	0.20
Plasma creatinine	0.17	0.15	0.14	0.15
Smoking – pack/yrs	0.02	0.01	0.01	0.01
Erythema index	-0.27 *	-0.25 *	-0.23 *	-0.09

* $p < 0.05$

- Figure 5 shows the correlation between the duration of diabetes and several derived ESAF values (mean and standard deviation of the increase in amplitude in each bin following enhancement, magnitude, offset, and root mean square). The correlation between diabetes duration and ESAF arbitrary units expressed as mean values or magnitude was greatest in bin 14 (467nm; $r = 0.23$; < 0.001). This significant relationship ($p < 0.05$) between these variables was observed in bins 8-28 (420-580nm).
- The standard deviation, offset and root mean square of the measurements showed similar but less significant patterns.
- ROC curve analysis of Prototype 7 data showed the ability of ESAF was statistically significant, but with less power than either HbA1c or non-fasting glucose concentration (Figure 6).

Figure 5. Correlation between duration of diabetes and derived ESAF variables in the combined data of the diabetic and control groups.

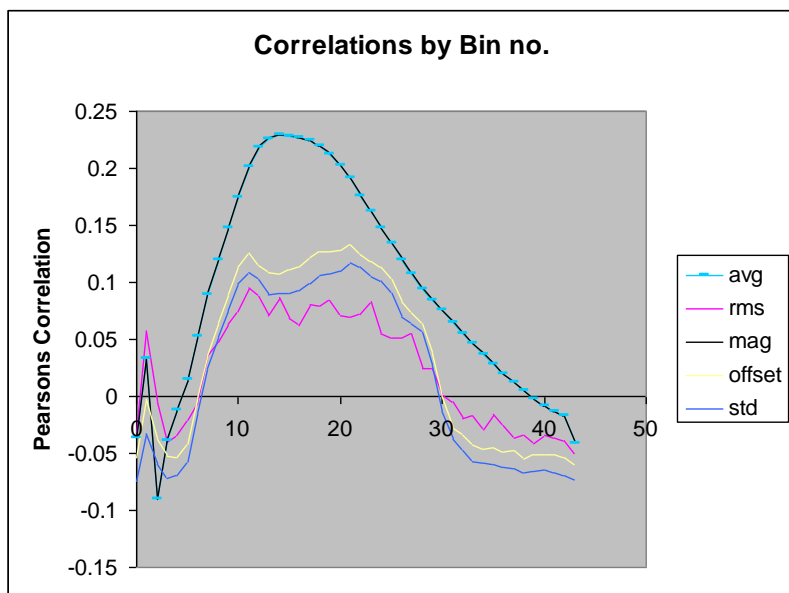
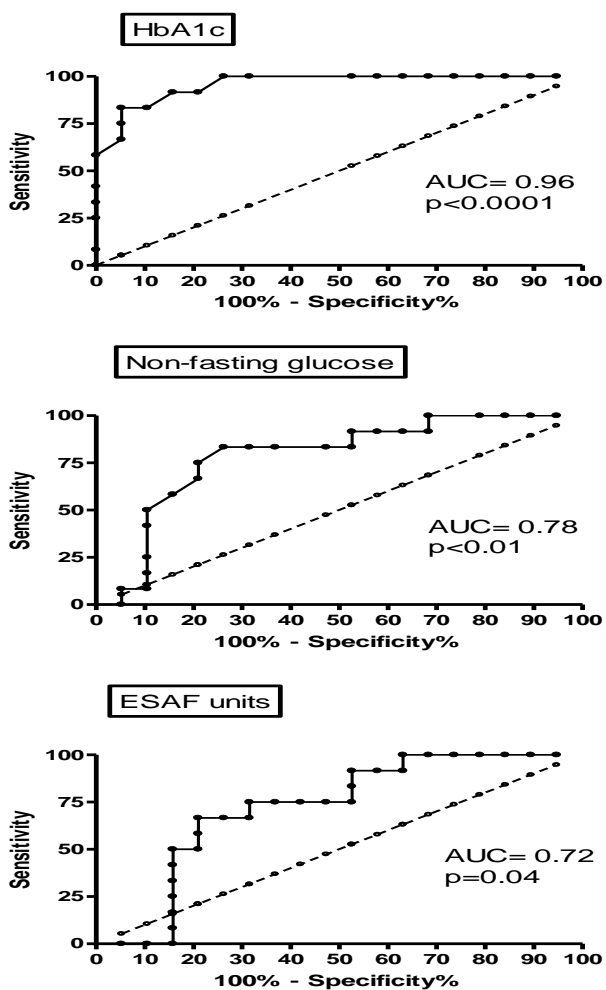


Figure 6. ROC curve analysis of ability of prototype 7 to discriminate between diabetes and non-diabetes compared to non-fasting glucose and HbA1c levels.



Prototype 8. September 2010 – February 2011

- This prototype used the same scan profile as prototype 7, in addition to simultaneous, internal measurement of skin colour using an LED light source. This addition was required as the data obtained with Prototype 7 showed skin colour was a major factor in the measurement of ESAF. A total of 106 subjects (64 diabetics, 42 controls) in the study database had repeat scans using Prototype 8 (Table 6).
- On November 18, 2010, the ESAF measuring device was relocated to MedLab South Laboratories, Christchurch to compare the ability of ESAF and an OGTT to detect diabetes (n=69) or gestational diabetes (n=71). ESAF would not be expected to be increased in gestational diabetes due to the short-term nature of the condition with values anticipated to be within the same range as normal controls.

Table 6. Clinical data of subjects scanned with Prototype 8.

Abnormally distributed data compared using Wilcoxin Rank Sum Test* and categorical variables using the Chi-square test#.

	Diabetes n=64	Control n=42	P value	Comparison with OGTT	
				Diabetes n=69	Gestational Diab n=71
Gender (M/F)	35/29	17/25	NS #	40/29	0/71
Age (yr)	61.5 (10.7)	54.9 (10.2)	<0.01	56.4 (12.8)	30.0 (5.87)
BMI (kg/m ²)	31.1 (6.2)	33.5 (6.9)	NS	30.1 (6.7)	-
Waist circumference (cm)	102 (17)	110 (15)	<0.05	-	-
Blood pressure (mmHg)					
Systolic	130 (13)	125 (24)	NS	-	-
Diastolic	81 (7)	83 (9)	NS	-	-
On antihypertensive agent (%)	53	47	NS #	19	2
Smoking history No/Ex/Current (%)	47/34/19	64/26/10	0.02 #	68/22/10	65/17/18
Pack years	14.0 (22.2)	9.5 (19.5)	NS *	6.7 (14.2)	1.8 (3.5)
Duration of diabetes (yr)	9.6 (8.8)*	-	-	-	-
Ethnicity (%)					
Caucasian	92	93		93	79
Maori/Pacific Island	6	7		5	11
Asian	2	0		1	10
Middle Eastern	0	0		1	0
Skin colour					
Erythema index	12.31 (2.87)	12.26 (2.18)	NS	12.83 (3.36)	9.53 (1.65)
Melanin index	34.26 (4.90)	32.79 (3.15)	NS	35.83 (8.67)	28.25 (3.36)
R	116.6 (12.7)	120.2 (8.6)	NS	113.3 (19.2)	133.4 (9.4)
G	88.5 (13.9)	91.0 (10.4)	NS	87.0 (21.8)	107.7 (10.3)
B	87.6 (15.1)	91.2 (10.8)	NS	84.9 (19.8)	106.8 (12.6)
Non-fasting glucose (mmol/L)	9.7 (4.4)	5.7 (1.2)	<0.001*	-	-
Fasting glucose (mmol/L)	-	-	-	4.4 (0.4)	5.9 (1.2)
+ 1 hr glucose (mmol/L)	-	-	-	7.4 (1.7)	10.8 (3.5)
+ 2hr glucose (mmol/L)	-	-	-	6.2 (1.4)	8.4 (4.1)
HbA1C (mmol/mol)	61.0 (17.6)	38.5 (4.5)	<0.001*	43.0 (6.5)	33.0 (3.9)
Haematocrit	0.44 (0.04)	0.44 (0.04)	NS	-	-
Plasma bilirubin (µmol/L)	8.4 (3.1)	9.5 (4.8)	NS	-	-
Prevalence vascular complications (%)					
Retinopathy	11	-		-	-
Neuropathy	9	-		-	-
Nephropathy	14	-		-	-
Peripheral vascular disease	5	-		-	-
Macrovascular disease	22	-		-	-

- The diabetes group was older, had a higher prevalence of smoking, and as expected, worse glycaemic parameters than the control group.
- Although the subjects screened for gestational diabetes by the OGTT had significantly lower HbA1c levels than the subjects screened for type 2 diabetes, their response to the glucose load was significantly greater at all time points.
- Data collection was ceased on February 22, 2011 as a result of the Christchurch earthquake. At present, the combined data of Prototypes 7 and 8 are being analysed with the objective of calculating a definitive algorithm for the ESAF method. This complex analysis is currently being carried out by Assoc Professor Chris Frampton, Statistecol, Auckland. The values derived by the algorithm will then be used for the comparative, ROC curve, correlation and cross-sectional analyses. The results of these analyses will be advised at a later date.
- Development of the algorithm for calculation of ESAF units includes:
 - Obtaining mean spectroscopic profile of 20 replicate scans in scans 1-4.
 - Dividing the wavelength spectrum (355-700nm) into \cong 8nm bands for each scan.
 - Standardisation of data to fluorescent intensity measured at 360nm.
 - Standardisation of data to skin colour variables.
 - Calculation of mean amplitude and offset of curve in each 8nm wavelength band.
 - Selection of bins and derived variables that show the strongest correlation with glycaemic indices.
 - Standardise for the effects of age and differences in haematocrit and bilirubin levels.

Summary and conclusions

- Further development of the ESAF method was achieved during the period of HRC funding. This development included incorporating a considerably more sensitive fluorospectrophotometer and refinement of the scan sequence to 1) standardise for interference from cutaneous vasomotion, 2) maximise the photochemical changes induced by enhancement with 254nmUV, 3) standardise for differences in skin colour, and 4) minimise the development of erythema during the scans.
- Data collection was started in April 2010 and completed in February 2011. Preliminary analysis of this data shows that the ESAF method has approximately similar ability as non-fasting glucose to discriminate between diabetes and non-diabetes, but less ability than HbA1c.
- There was a strong correlation ($p < 0.001$) between ESAF arbitrary units measured at 467nm and duration of diabetes and mean HbA1c levels over the preceding five years. This indicates the ESAF method is a sensitive marker of glycaemic load. Whether or not the method has sufficient sensitivity to identify undetected diabetes has yet to be established. Completion of the comparative analysis between the OGTT and ESAF method will provide information regarding this question.
- Our results are similar to those reported by VeraLight, a USA-based company that manufactures a commercial AGE reader for non-invasive screening of type 2 diabetes (6). Their studies showed that SAF did not correlate with a single measure of recent HbA1c, but did correlate with chronic glycaemic exposure, as assessed by mean HbA1c over time.
- If sufficient sensitivity is achieved to discriminate between diabetes and non-diabetes, the non-invasive nature, lack of fasting requirement, relatively short analytical time, and low cost of ESAF may provide a first line screening method for diabetes.
- Further development of the scanning device is planned that will incorporate a shielded unit that will be attached to the subject's arm and interfaced by a fibre optic cable to the spectrophotometer and computer.

- The ESAF method also provides information on cutaneous vasomotion. Control of vasomotion is abnormal in patients with diabetes and may contribute to the development of neuropathy and peripheral vascular disease. An assessment of cutaneous vasomotion may be a clinically useful adjunct in the ESAF method.

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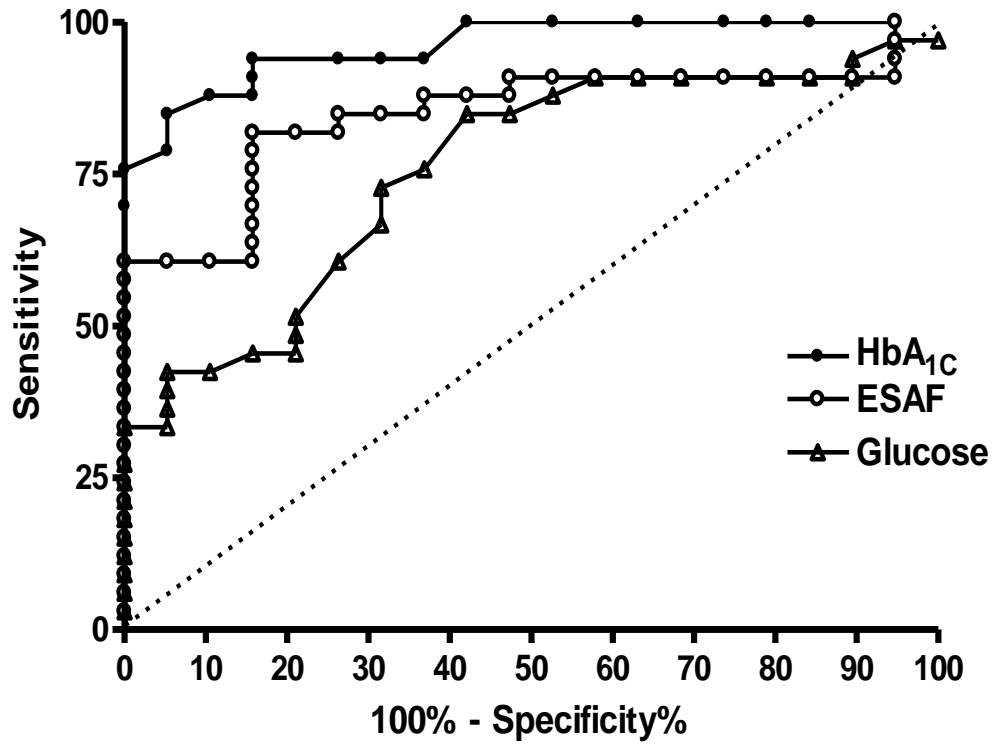
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Appendix 1

Comparison of the ability of prototype 1 (pilot study) to discriminate between diabetes and non-diabetes.

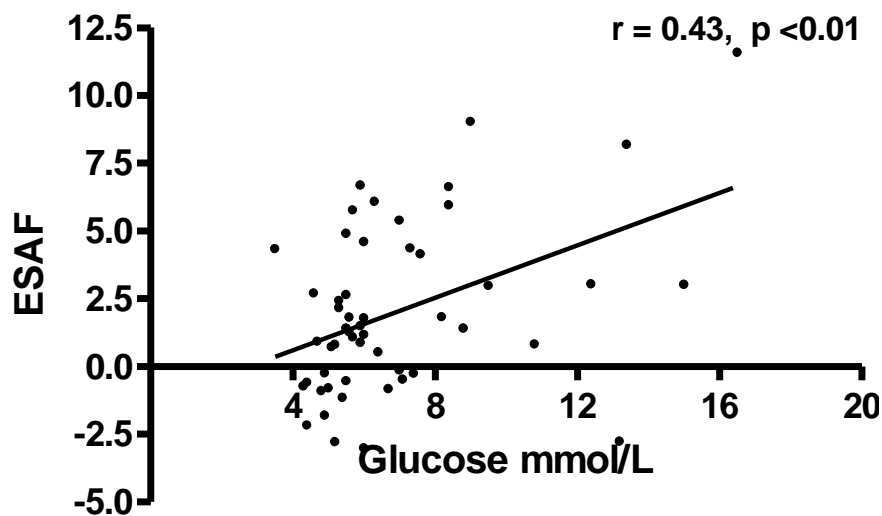
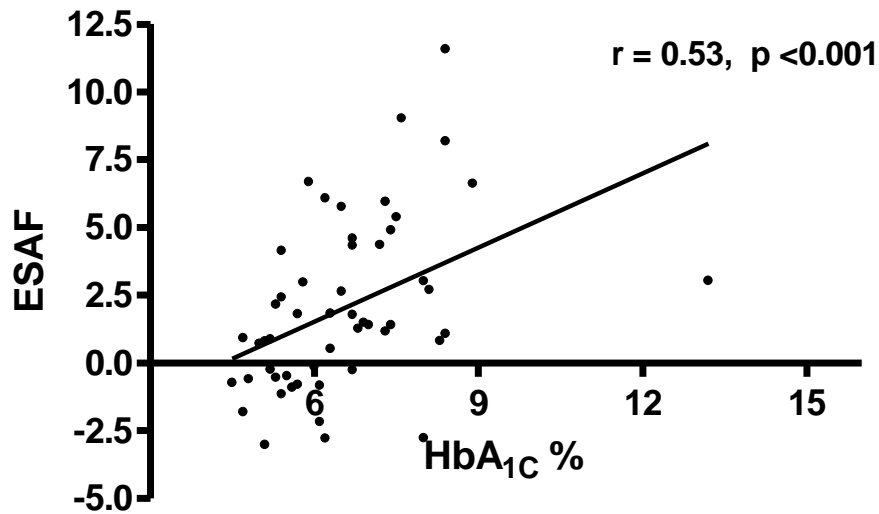
ROC curve analysis



Area under the curve \pm SEM

HbA _{1c}	0.96 ± 0.02	$p < 0.0001$
ESAF	0.85 ± 0.06	$p < 0.0001$
Non-fasting glucose	0.76 ± 0.07	$p = 0.002$

Correlation between ESAF measured by Prototype 1 (pilot study) and HbA1c or non-fasting glucose.



Appendix 3

Summary of findings of pilot study (Prototype 1)

- ESAF measurements were significantly higher in patients with diabetes compared to controls.
- There was an overlap in ESAF measurements between diabetes and non-diabetes.
- ESAF correlated with non-fasting glucose levels, HbA_{1c} and skin colour.
- ESAF had less power than HbA_{1c} but greater power than non-fasting glucose concentration to discriminate between diabetes and non-diabetes.
- Requirement to increase the sensitivity of the ESAF method.