

# Measurement of HbA1c in blood spot samples by a novel ELISA – for assessing the diabetic status of patients in resource limited settings

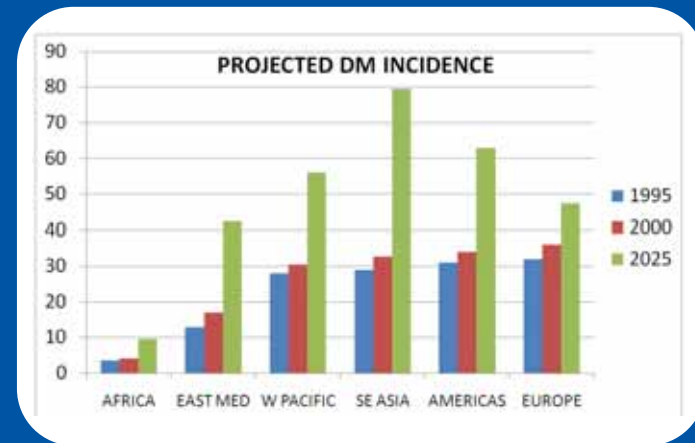
## AIM

In many parts of the developing world transportation of blood samples is a problem and sophisticated testing equipment only found in a few major centers. To facilitate blood testing for HbA1c in these situations a blood spot assay has been developed.

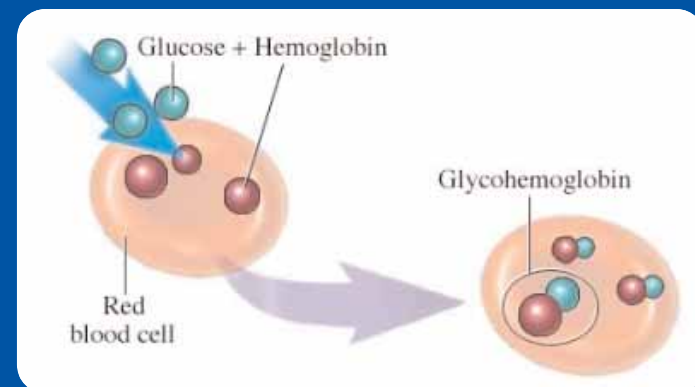
## INTRODUCTION

Diabetes mellitus, once thought to be uncommon in the developing world, has now emerged as a serious public health problem in Asia<sup>1</sup>. Pakistan is one of the 10 countries in the world with the highest prevalence of diabetes and has one of the fastest increases in the number of diabetics.

For this reason, this study was conducted by SCIPAC in conjunction with scientists from the National Health Research complex in Lahore, Pakistan.



HbA1c measurement is accepted as the best guide to discovering and monitoring diabetic patients. Glucose attaches to red cell hemoglobin to form glycohemoglobin. The level of glycohemoglobin formed is in proportion to medium term blood glucose levels.



## THE PROBLEM

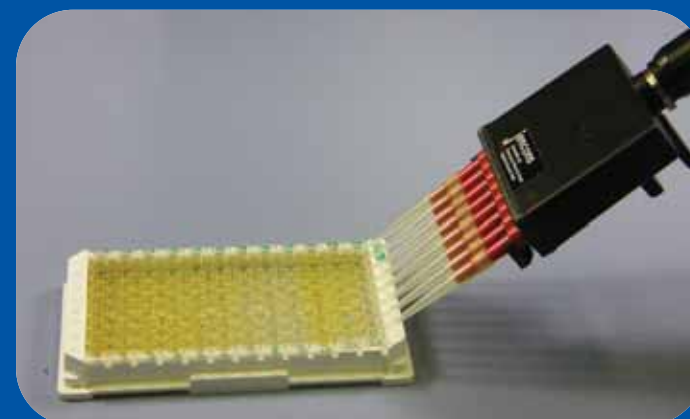
In many developing countries there is a shortage of trained phlebotomists and testing equipment is not found in rural areas. An alternative is to use blood spot samples. These make for ease of collection and distribution from widespread rural populations. Samples can be collected in the field and sent by post to central laboratories for elution and testing. The blood spot samples on filter paper are stable for at least 7 days.



## STUDY DESIGN

A high sensitivity ELISA was developed with optimal binding of hemoglobin (glycated and non-glycated)<sup>2</sup>. Microtitre plate wells were coated with purified haptoglobin and a signal produced by a HRP conjugated monoclonal antibody, specific to the glycated region of the HbA1c.

The assay was calibrated using stabilized HbA1c solutions and the assay was validated using external controls.



To enable verification of the method, samples of whole blood were collected in EDTA tubes and spotted on specially prepared filter paper and allowed to dry. After several days samples of blood spots were eluted with a stabilizing buffer solution, lysed and tested in the ELISA. The ELISA result was compared with results from whole blood samples measured by HPLC on the Bio-Rad Variant II.

## METHOD

HbA1c Blood spot ELISA using Microtitre plate:

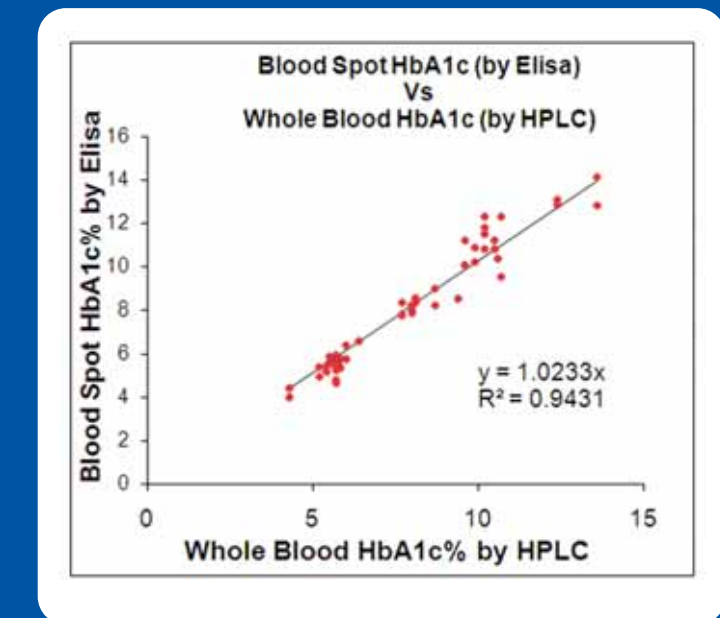
- Blood spots collected on filter paper, dried and stored several days.
- 6mm discs were punched out of each spot.
- Blood spot eluted in Buffer + 0.1% Triton.
- Eluted blood spots were added to a Microtitre plate well.
- Standards prepared from SCIPAC purified material.
- Anti-HbA1c-HRP conjugate added and incubated.
- Washed with 0.01% Triton-X-100.
- TMB Substrate added.
- Incubated and reaction stopped with 1M H<sub>2</sub>SO<sub>4</sub>
- Read at 450nm, subtracting 650nm.

## AUTHORS

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## RESULTS

Results from 54 diabetic patients were assessed using the two methods. The results for the blood spots produced a highly significant correlation with HPLC results (R<sup>2</sup> = 0.946).



## CONCLUSIONS

- A sensitive blood spot collection method for HbA1c has been developed which is easy to use
- Results are accurate and correlate very well with HPLC results
- The microtitre plates can be prepared locally keeping costs low
- The ease and cost effectiveness of this method will enable samples to be collected in the field and tested in central laboratories.

## References:

1. Global Prevalence of Diabetes, Diabetes Care, 27:5 (2004)
2. Non-separation assay for glycohemoglobin, Clin Chem 44:6, pp1302-1308 (1998)
3. Dried blood spot measurement of glycosylated hemoglobin. National Social Life Health & Aging Project (2008)