

# HPLC-based measurement of HbA1c using DBS collected under adverse field conditions

Duncan Thomas, Duke

Teresa Seeman, UCLA

Alan Potter, Univ Washington

Peifeng Hu, UCLA

Eileen Crimmins, USC

Henny Herningtyas, Univ Gadjah Mada

Cecep Sumantri, SurveyMeter

Elizabeth Frankenberg, UNC

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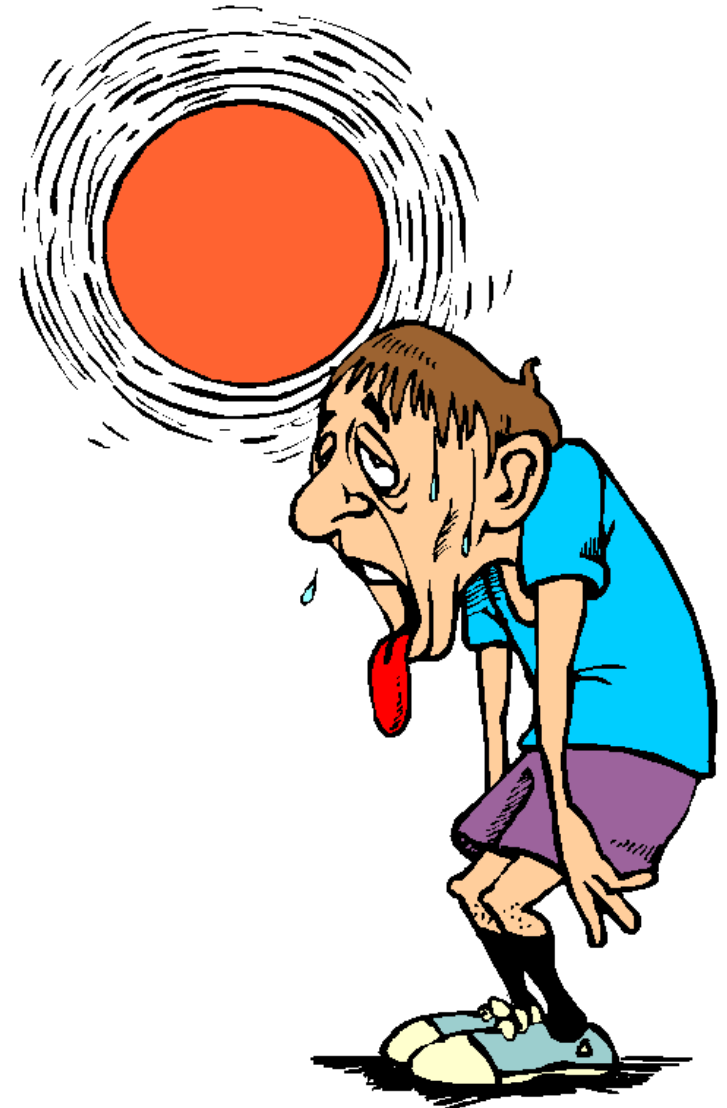
Forthcoming in *Biodemography and Social Biology*

**Goal:** Measure HbA1c in DBS  
collected under adverse conditions

What do we mean by adverse?

Hot and humid  
( $>85^{\circ}\text{F}$  and  $>70\%$ )

Why work under adverse conditions?



**Goal:** Measure HbA1c in DBS  
collected under **adverse conditions**

Part of larger project

**Study of Tsunami Aftermath and Recovery (STAR)**

Effects exposure to stress on cardio-metabolic health

3 key features: 1. Over **long term** (after 10y)

2. **Stress exposure exogenous**

Large scale natural disaster

2004 Indian Ocean tsunami  
(5% died, 15% displaced)

3. Pre-tsunami baseline pop<sup>n</sup>—  
representative, follow ups  
**re-interview 98% resp**

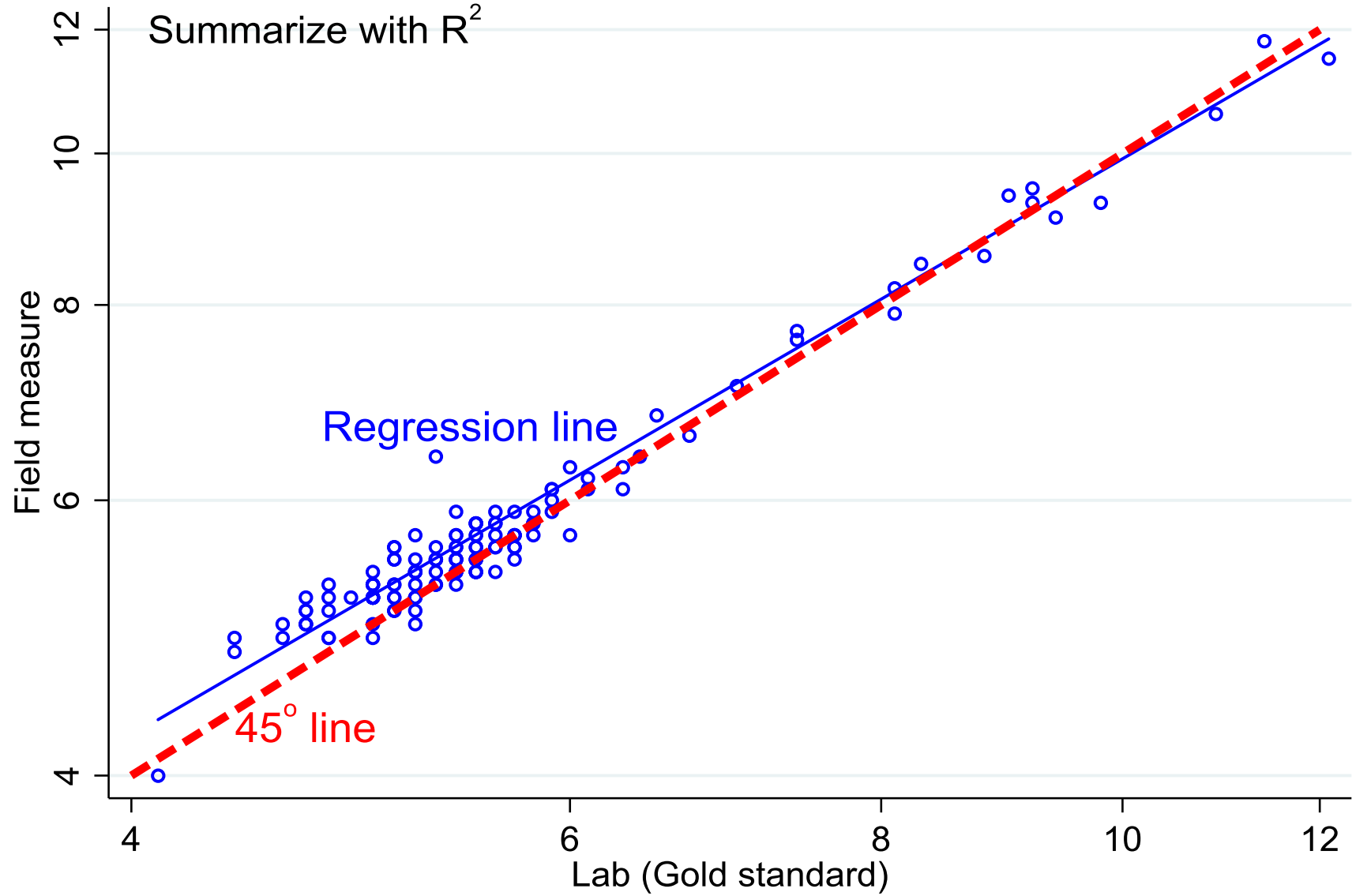


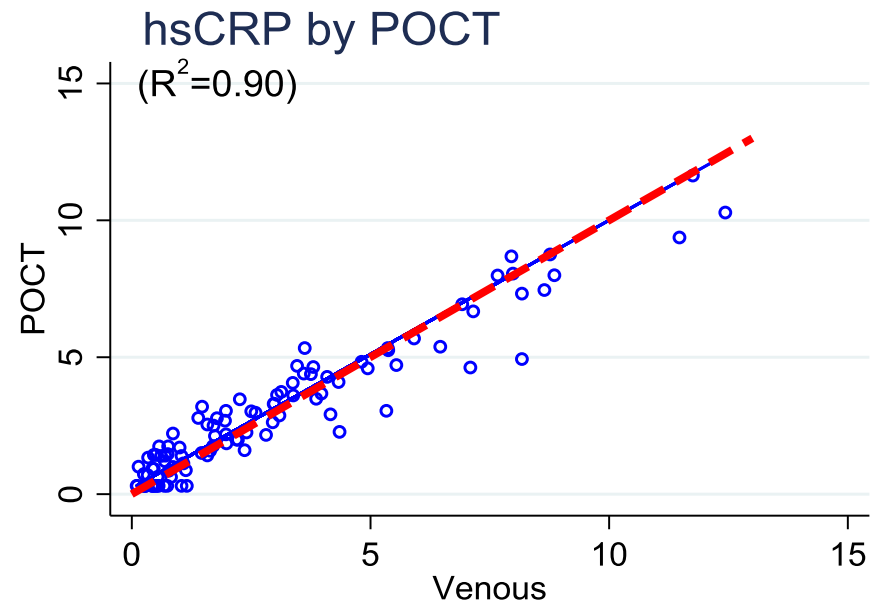
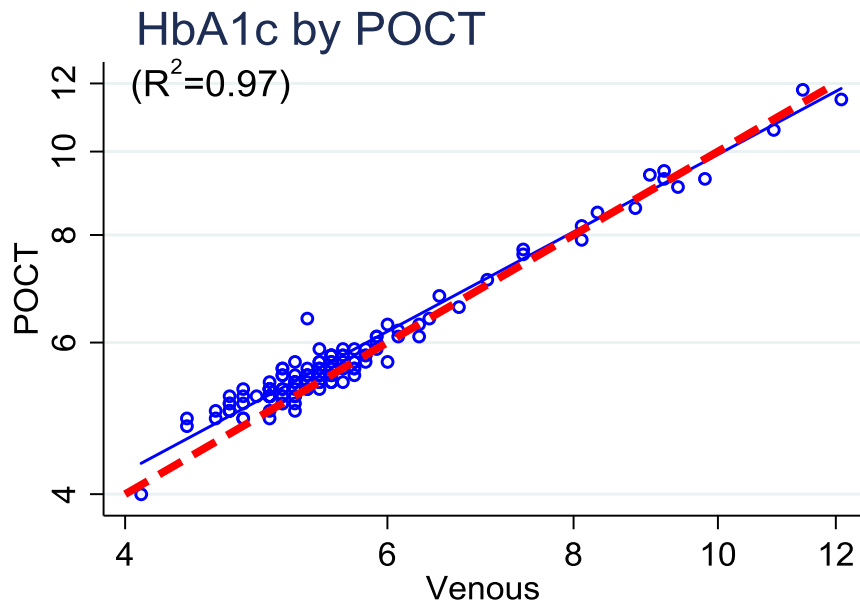
**Lessons likely relevant in many settings**

# Results from validation study

# Compare paired field and lab measures

Summarize with  $R^2$

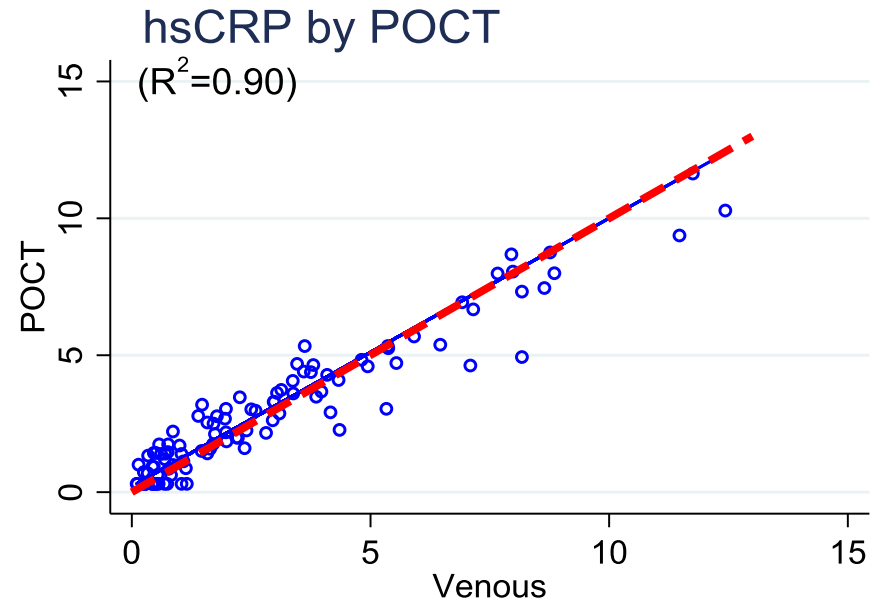
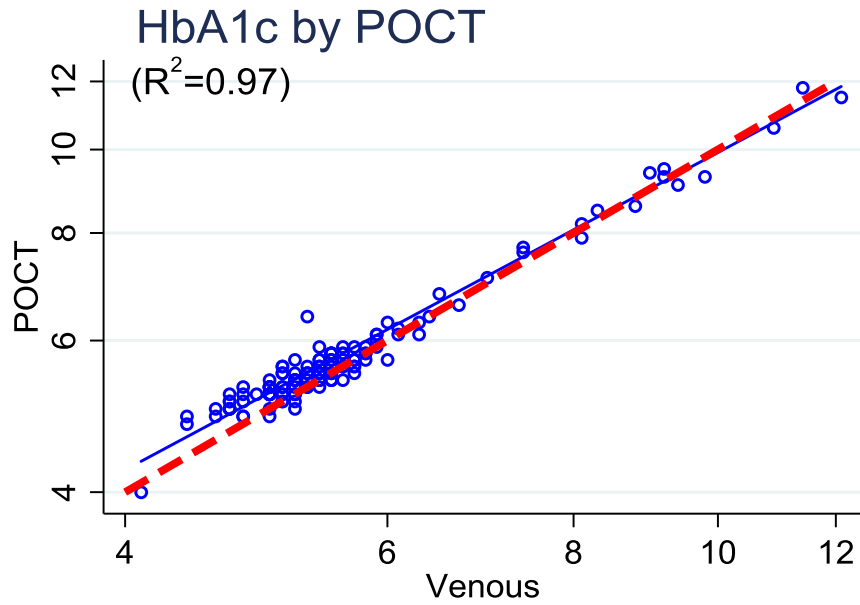




Validated Point of Care tests (POCT) against venous draws assayed in lab (gold standard) using local commercial lab  
HbA1c, hsCRP (and cholesterol)

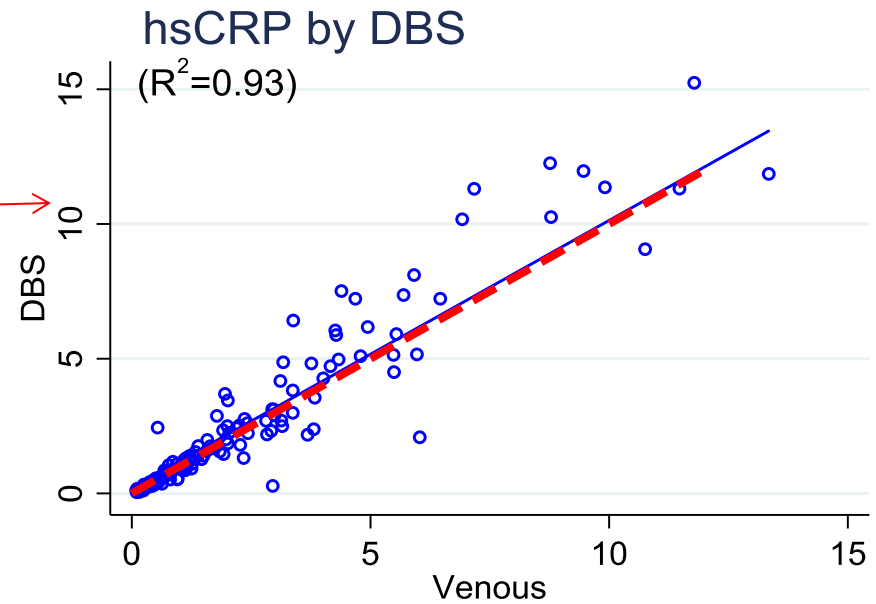
At same time created DBS  
assayed HbA1c and hsCRP

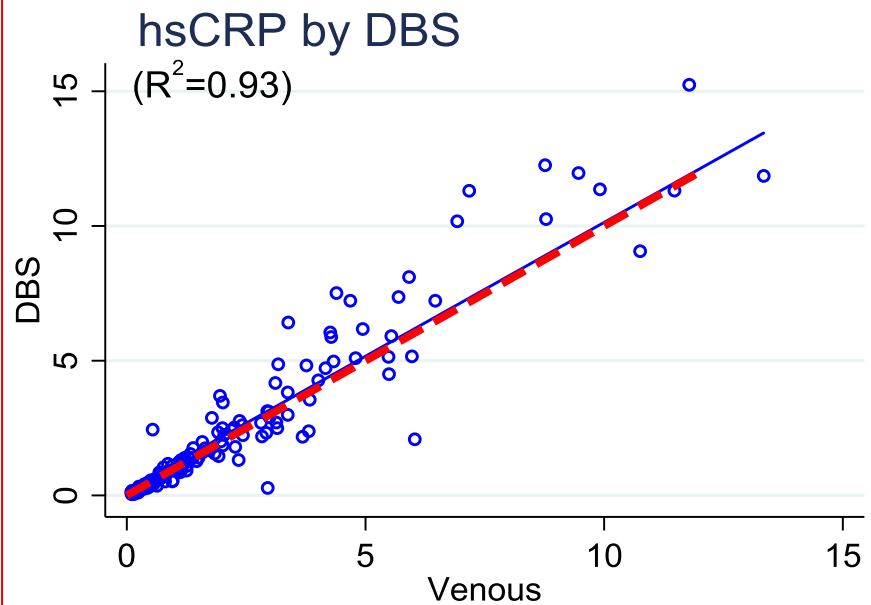
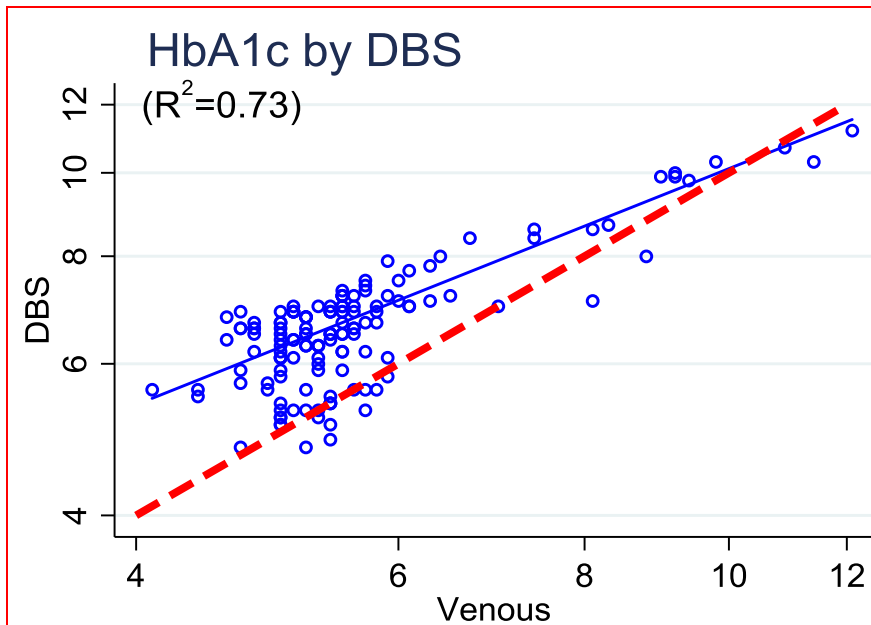
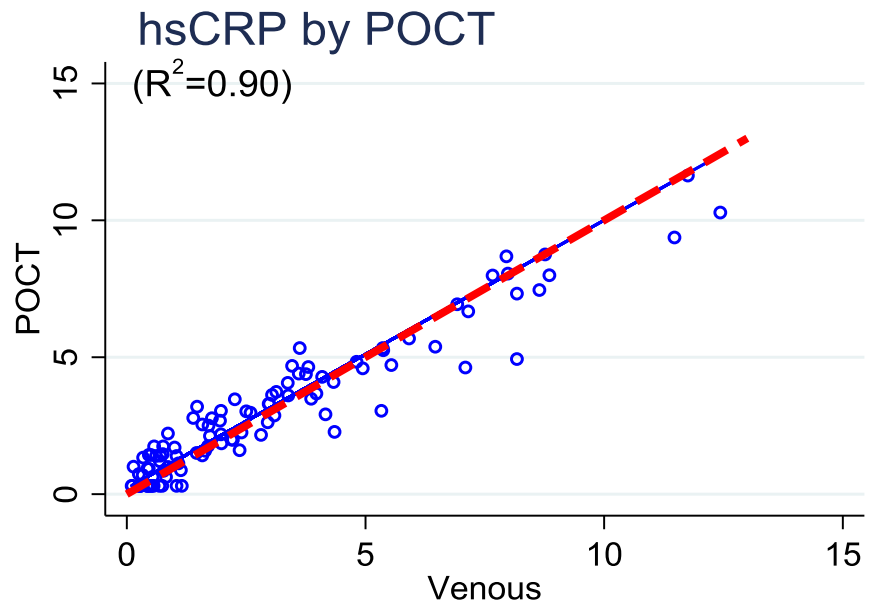
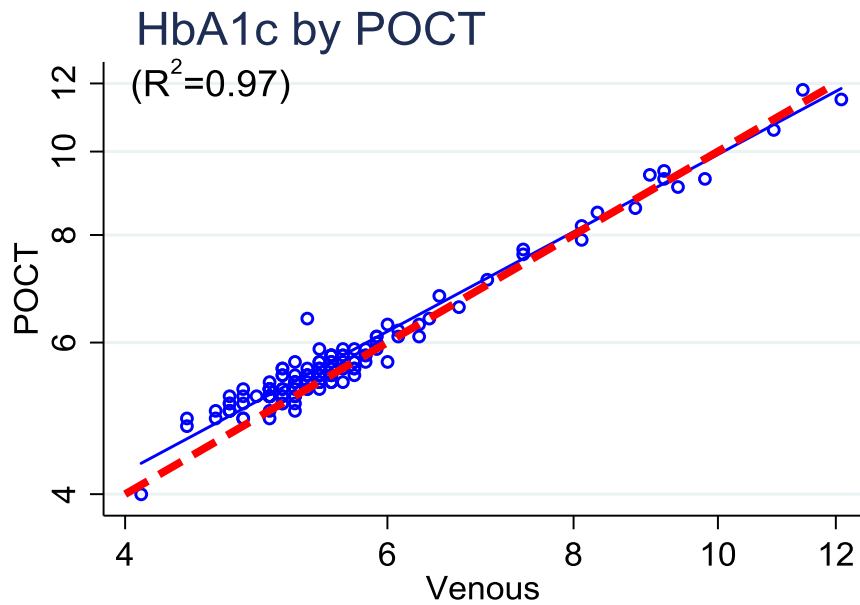




Created DBS and assayed at  
Herningtyas lab in Yogyakarta

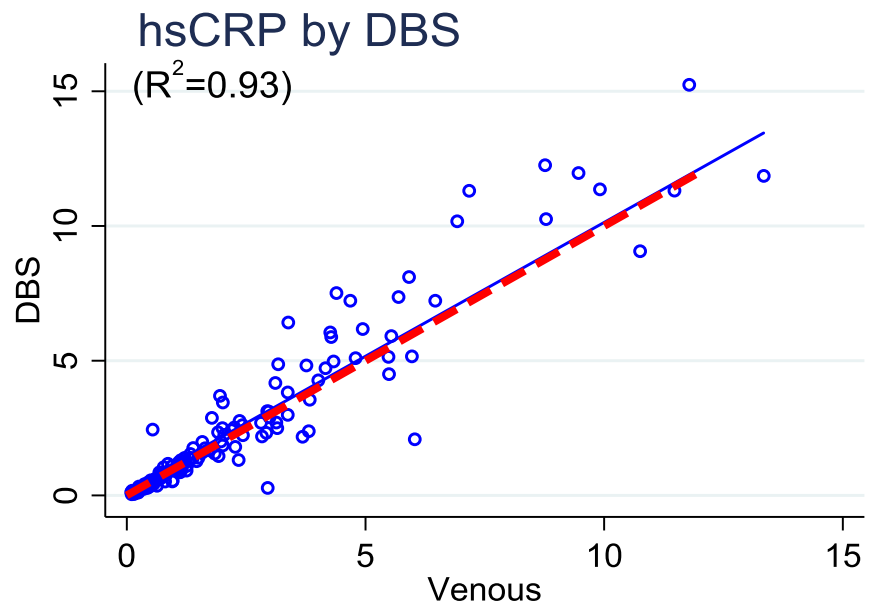
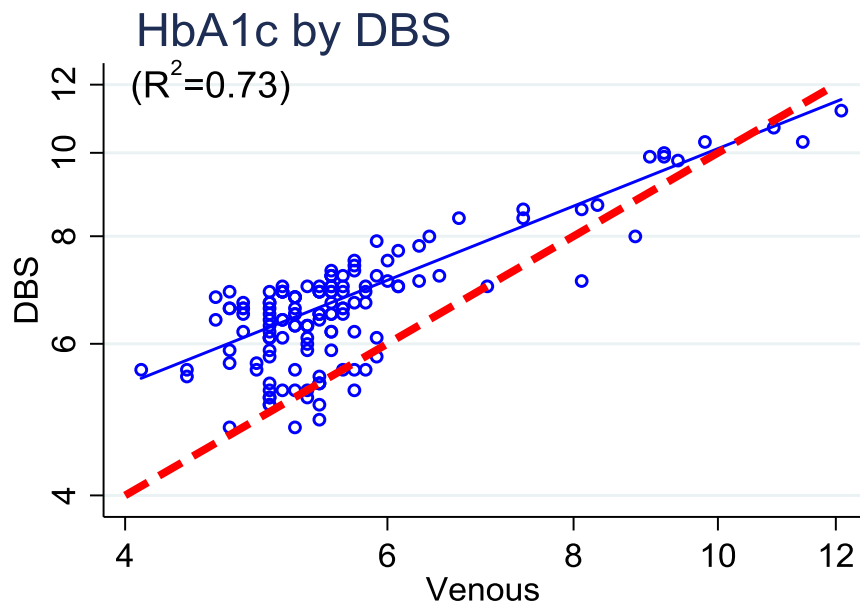
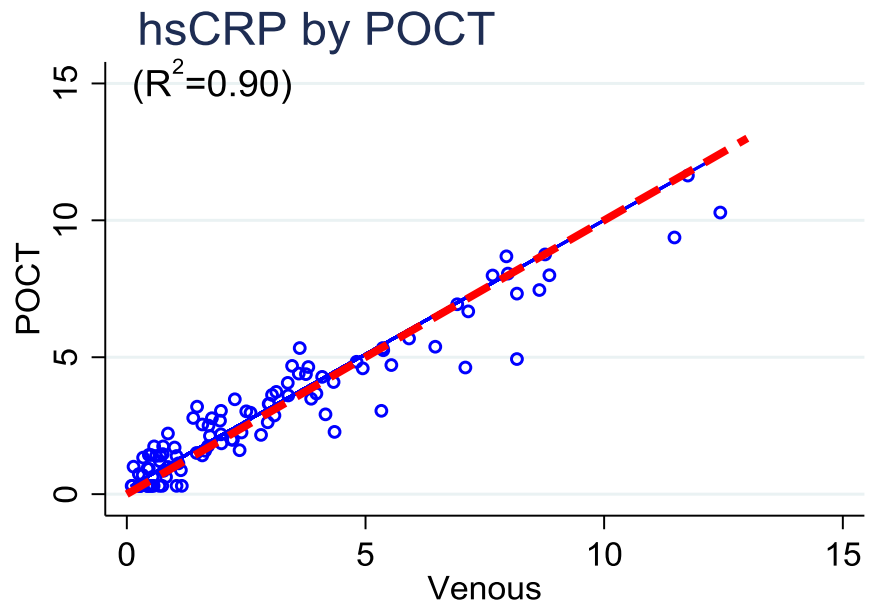
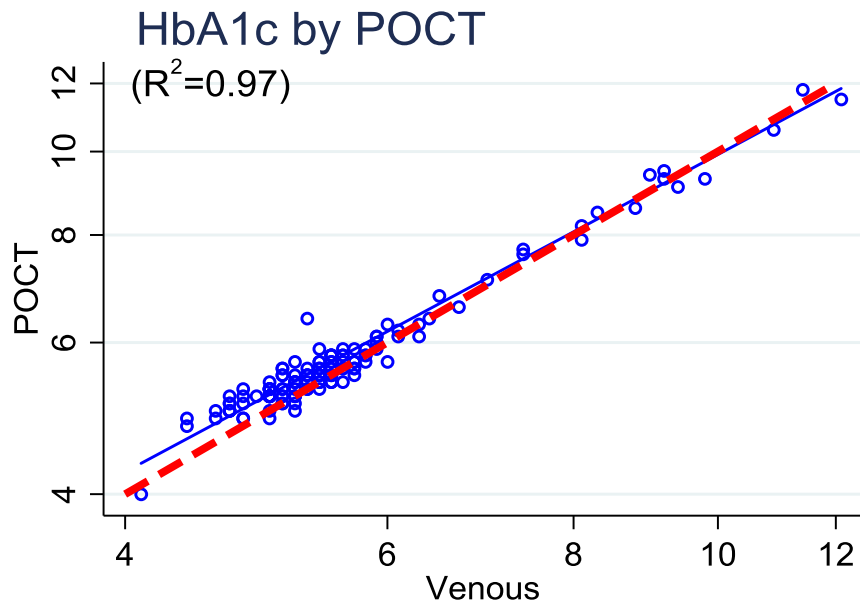
hsCRP performed very well



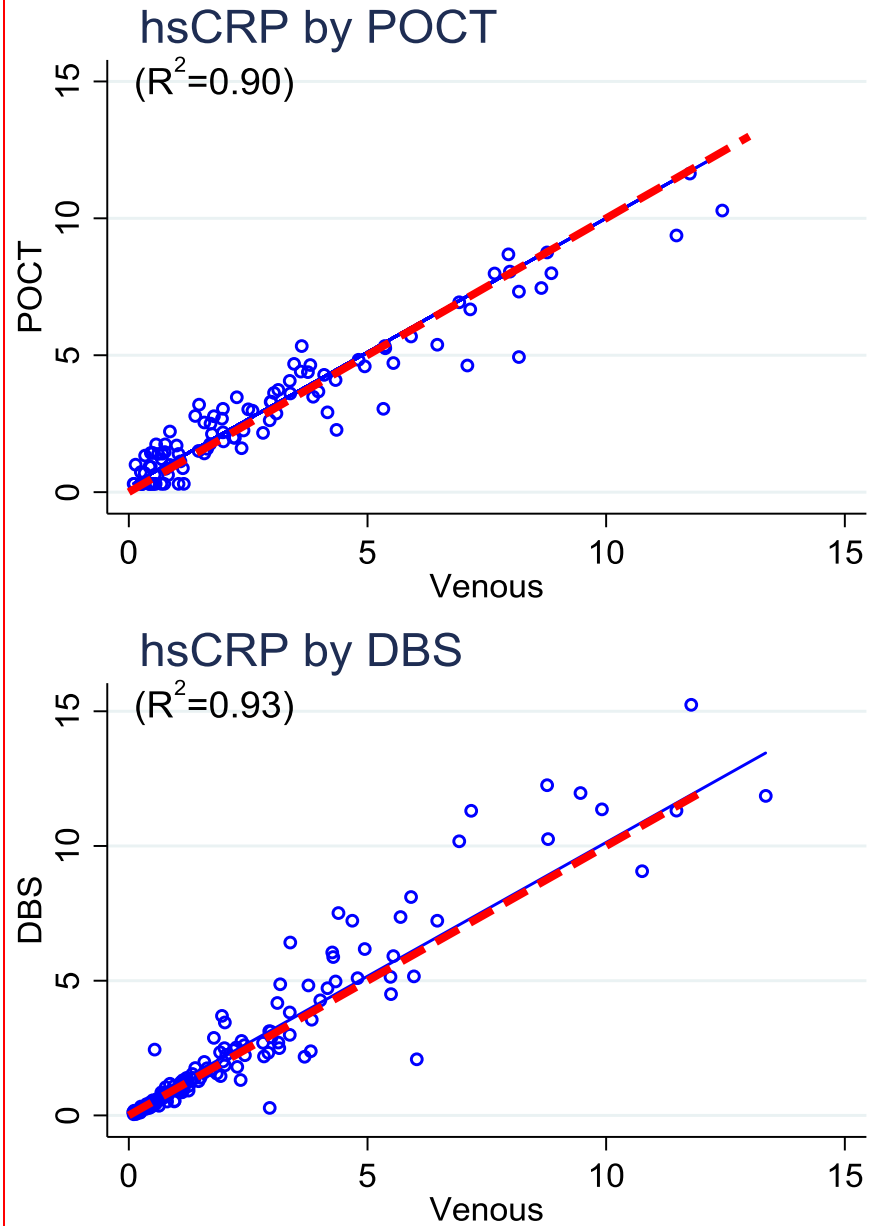
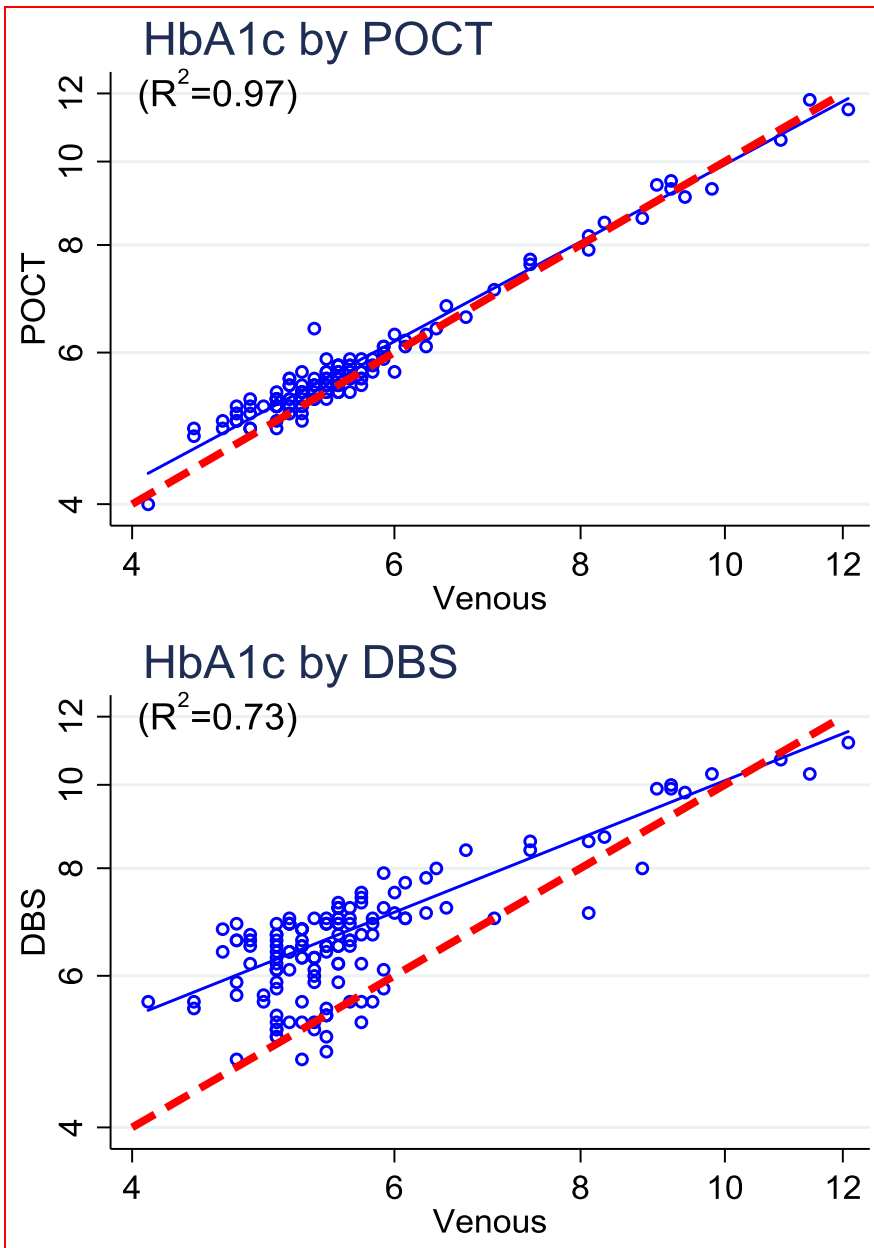


but HbA1c from DBS did not  
upward bias at low A1c, and very noisy

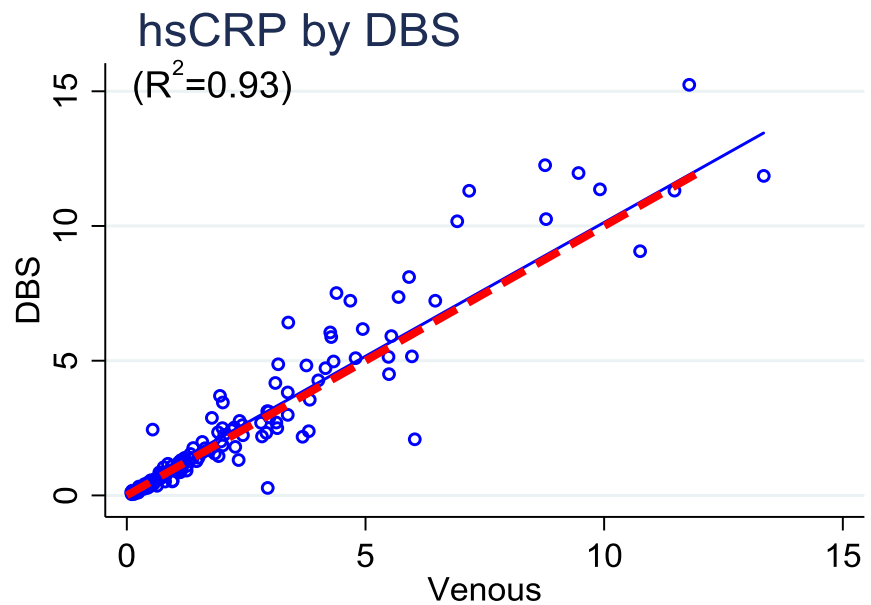
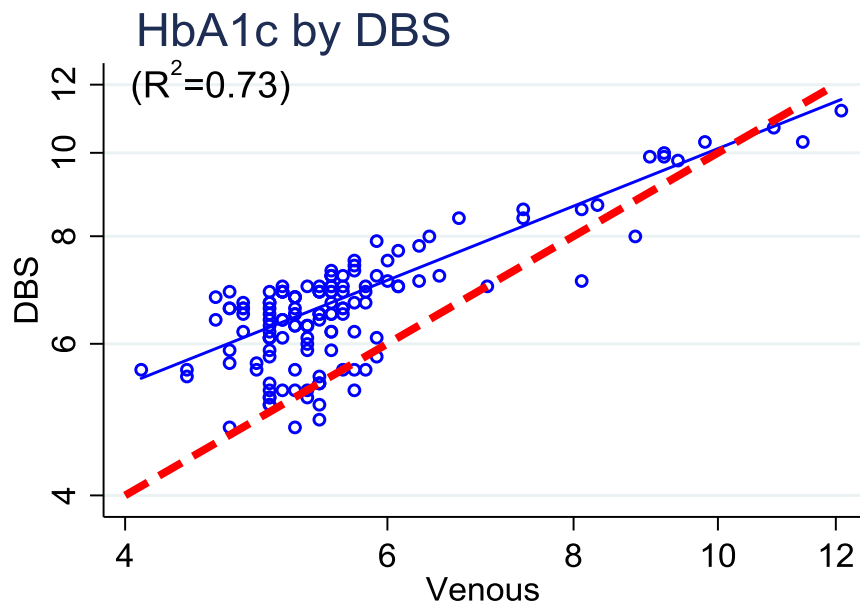
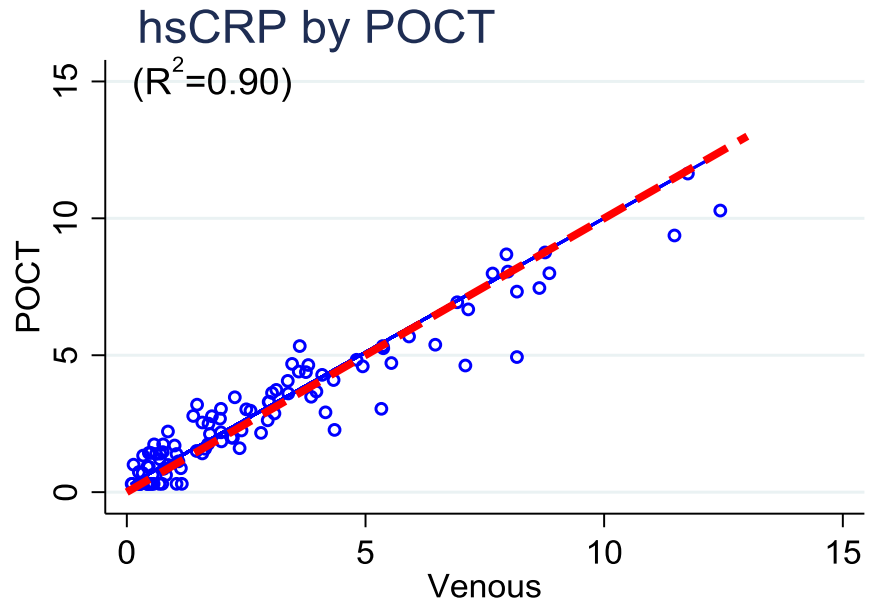
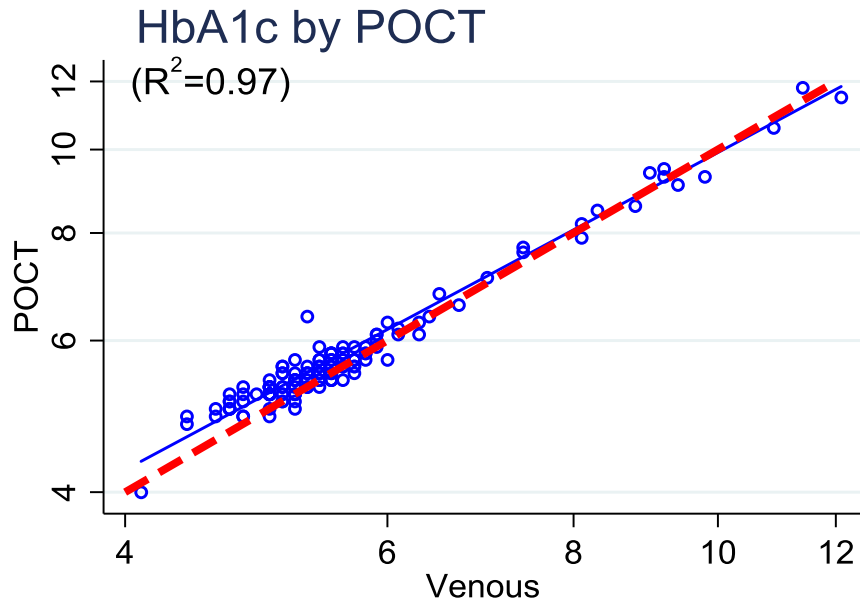




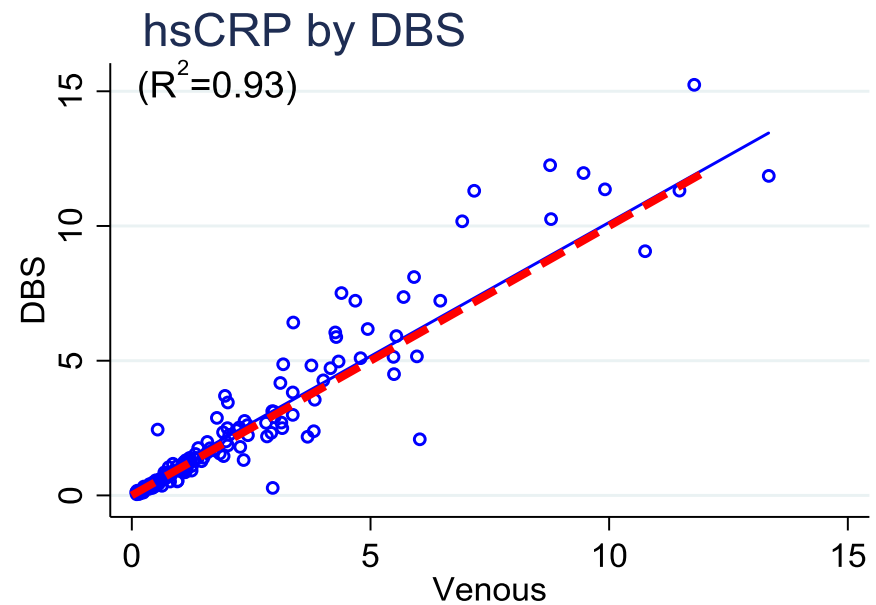
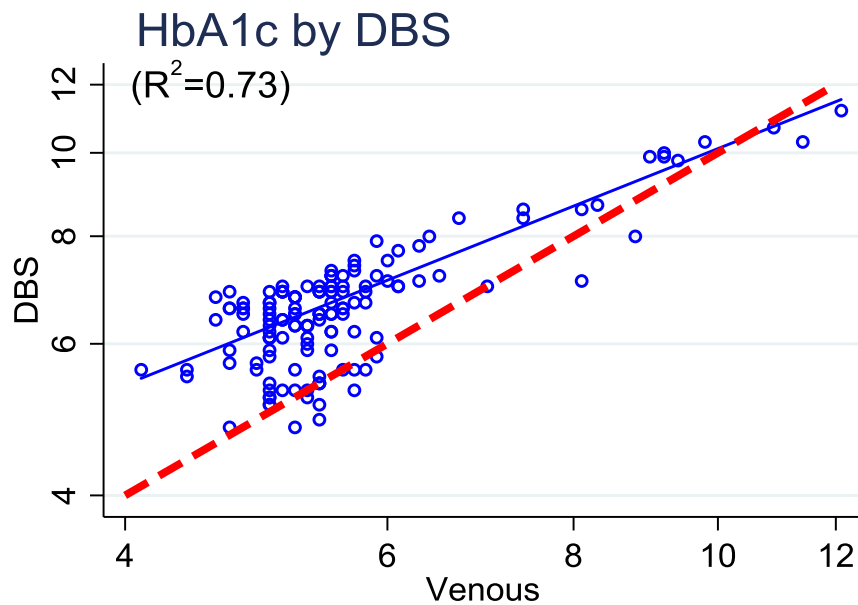
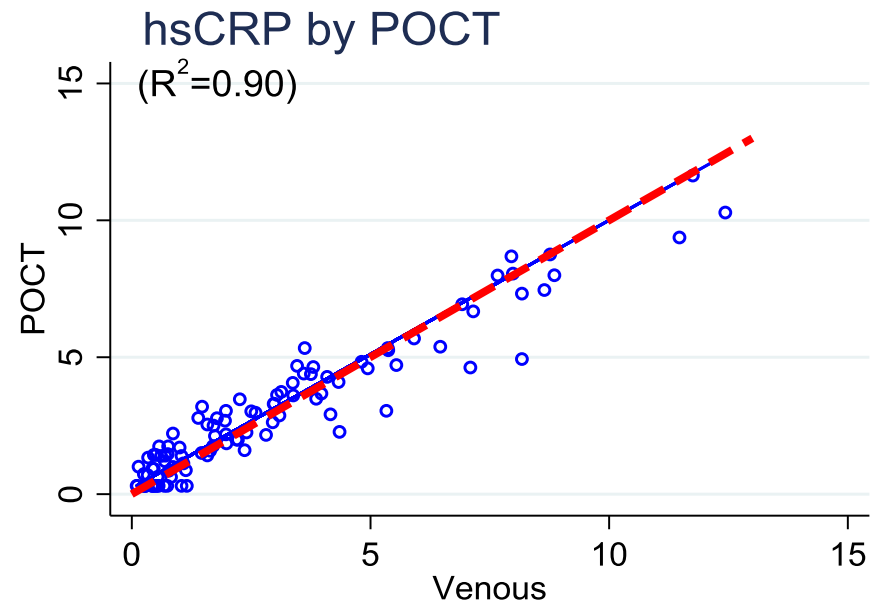
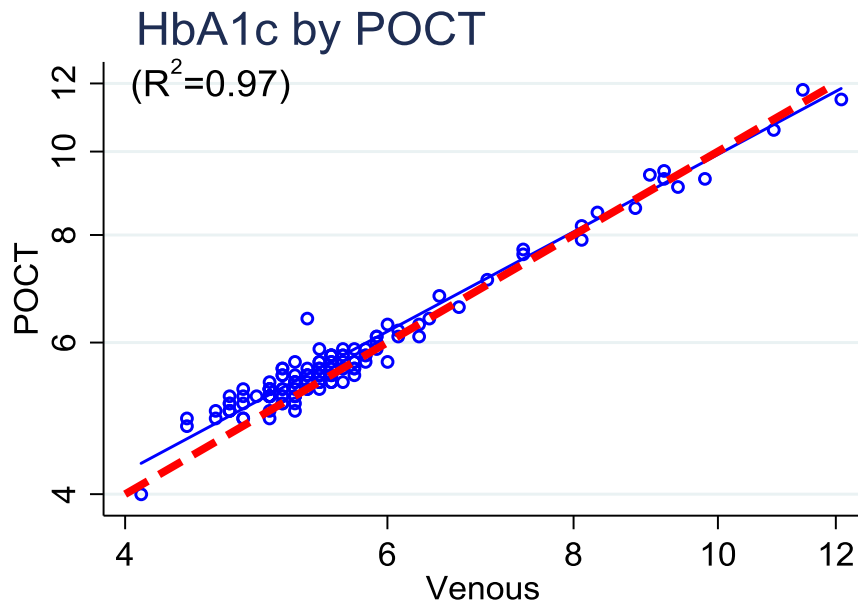
Maybe gold standard venous values wrong –



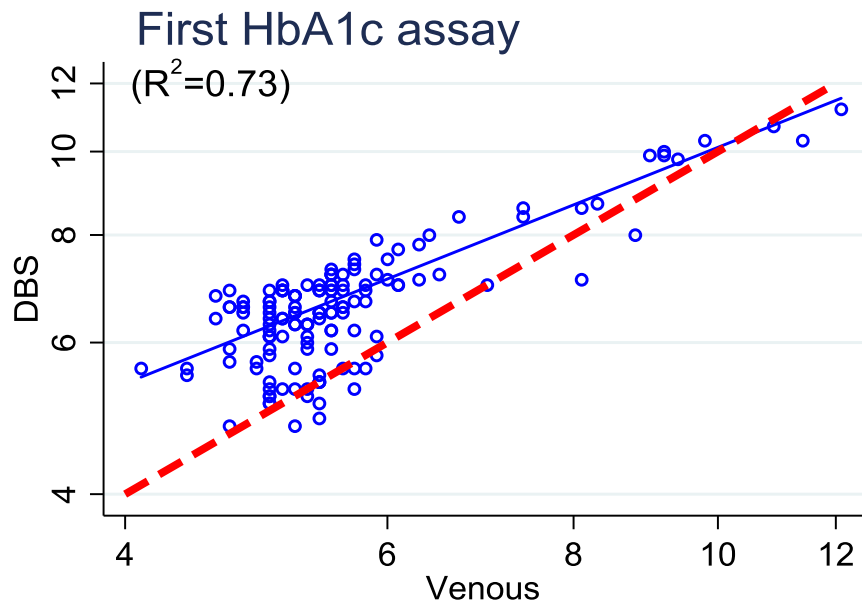
Maybe gold standard venous values wrong –  
unlikely since venous and POCT same



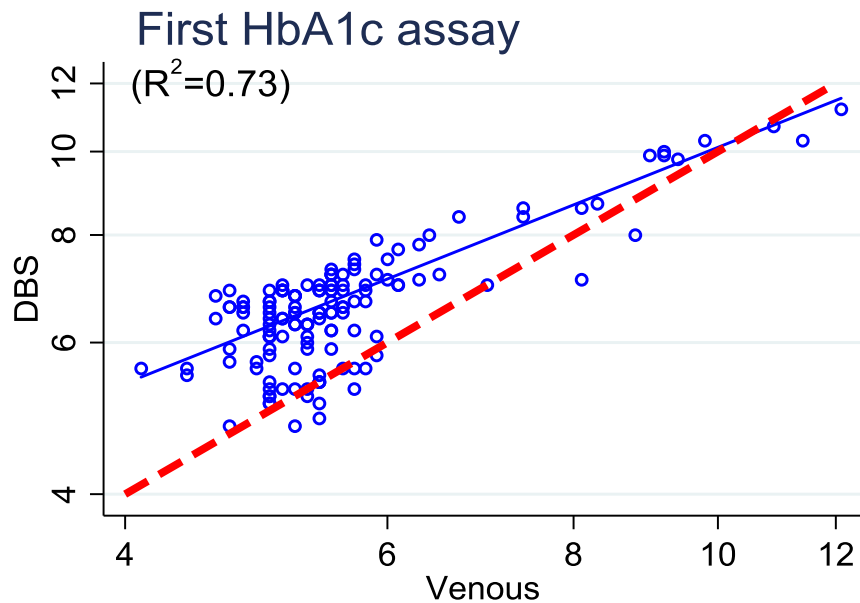
Maybe DBS compromised  
(collection, drying, storage)



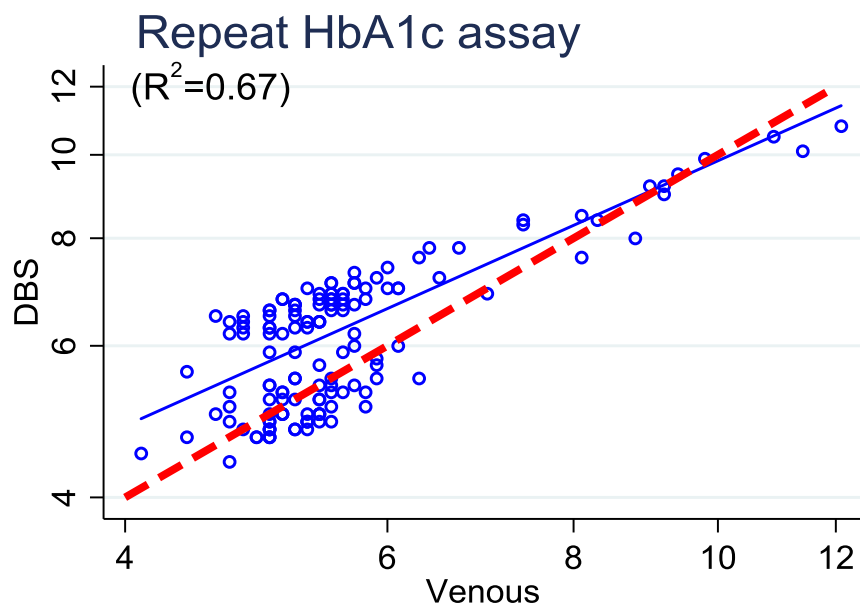
Maybe DBS compromised – unlikely since hsCRP DBS performed well (collection, drying, storage) (or problem is specific to HbA1c)



Maybe DBS A1c assays compromised



Maybe DBS A1c assays compromised  
(validated against Potter's lab)

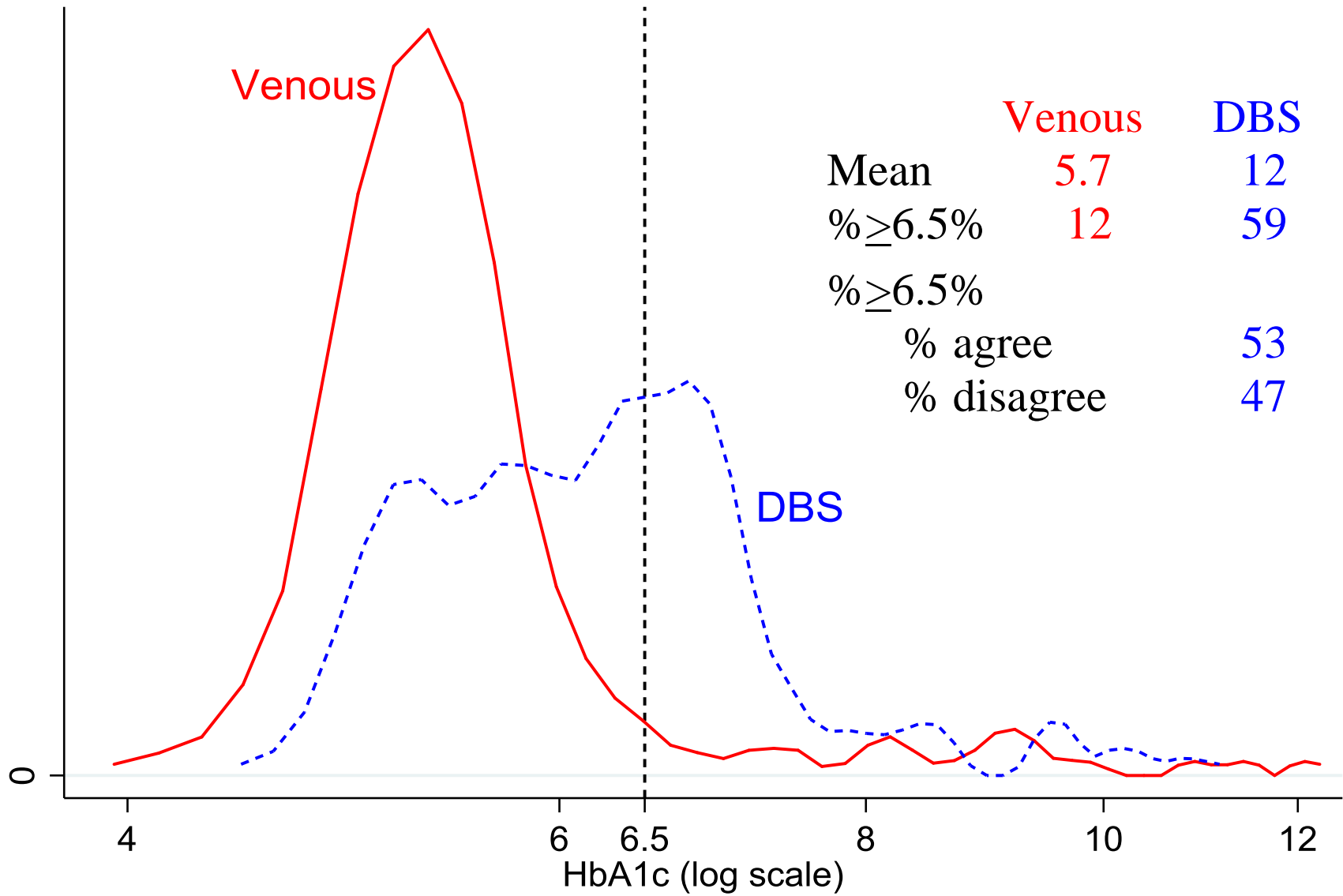


Reran DBS assays in Yogyakarta

Not substantially different

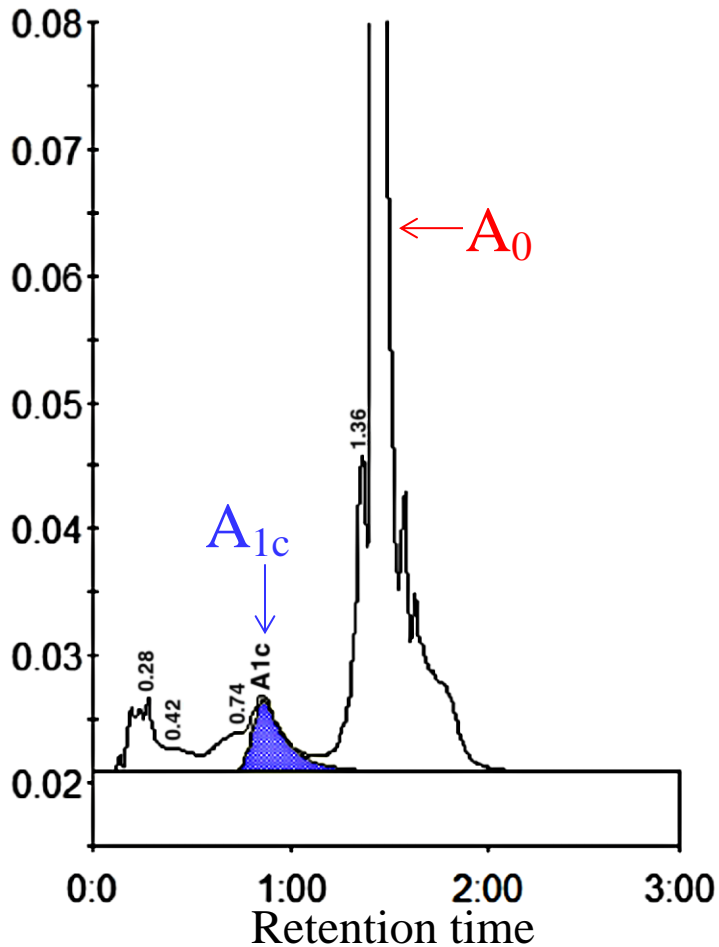
How different are the  
HbA1c distributions?

# HbA1c distributions: Venous and DBS





# D-10 HbA1c chromatograms



Bio-Rad D10 HPLC used for  
venous (gold std) & DBS assays  
Separates Hb components

Plots concentration of each Hb  
component against retention time  
in chromatogram

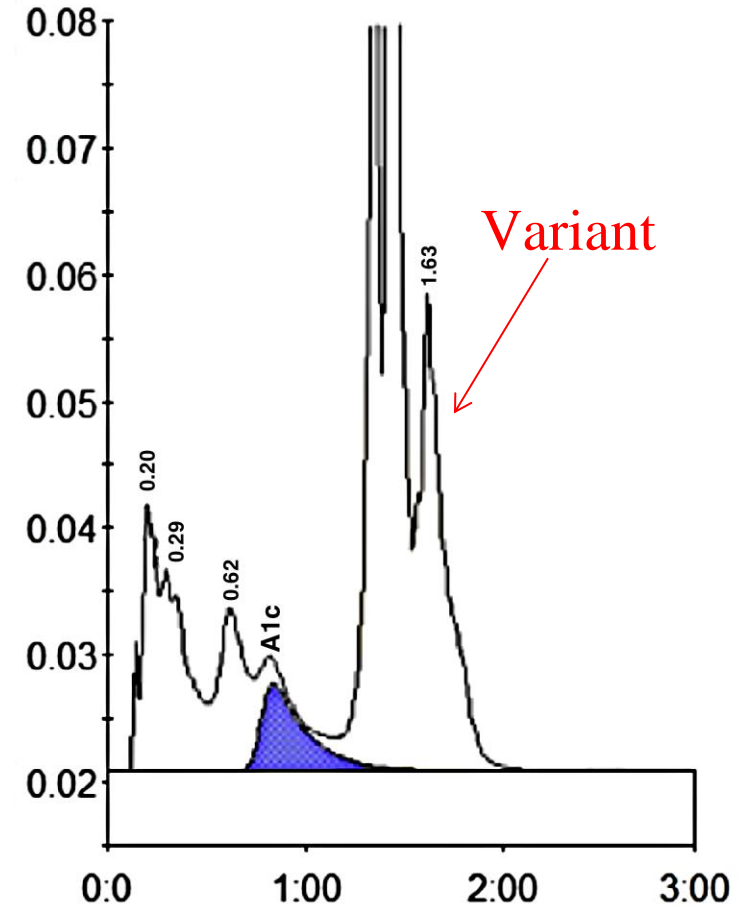
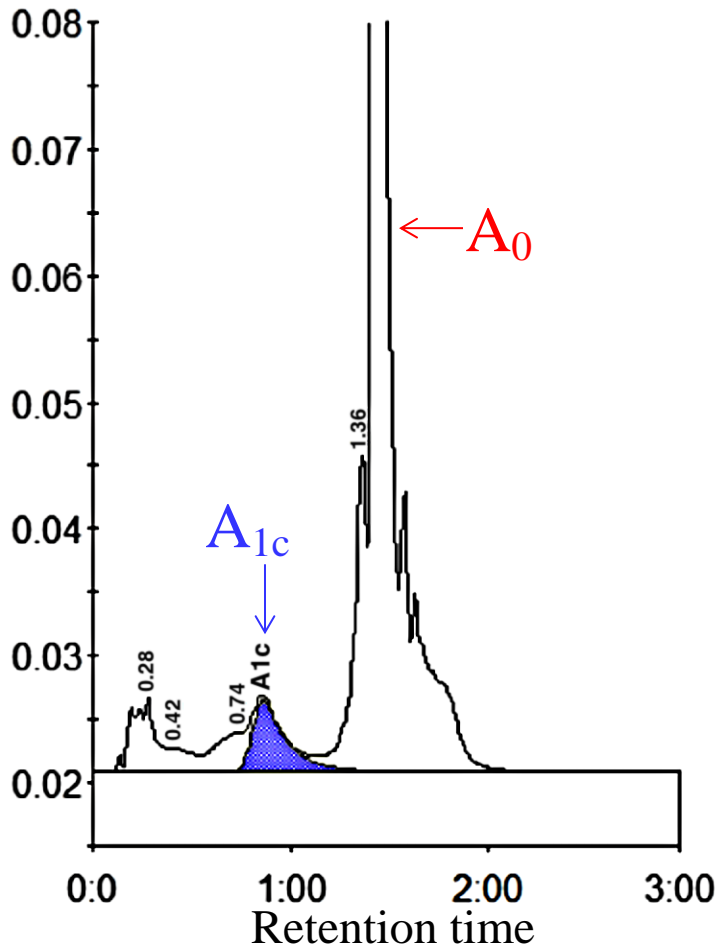
$$\% \text{HbA}_{1c} = \frac{\text{Area under } A_{1c}}{\text{Total Area incl } A_0}$$

Reports % HbA<sub>1c</sub>

# D-10 HbA1c chromatograms

A. Well-determined

B. Inspection of some of our DBS



$$\% \text{HbA}_{1c} = \frac{\text{Area under } A_{1c}}{\text{Total Area incl } A_0}$$

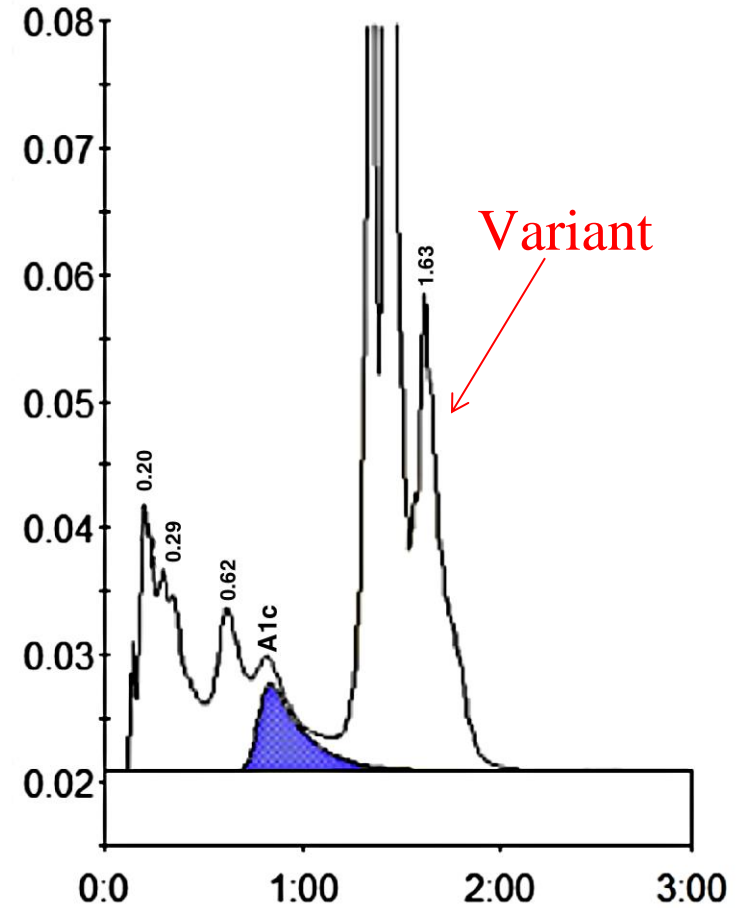
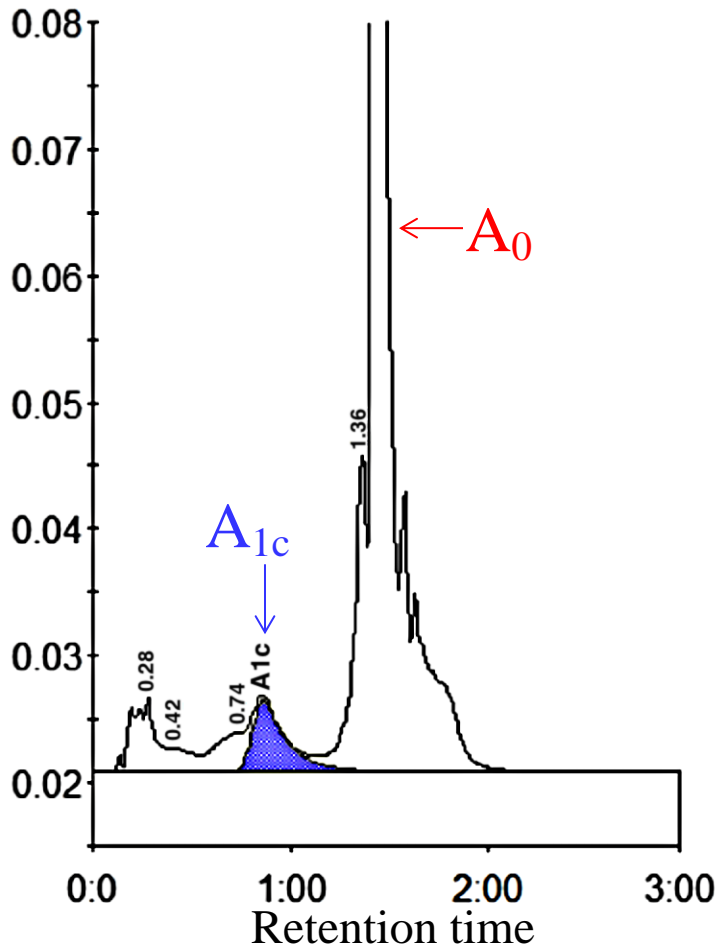
Peak to right of  $A_0$  misidentified as sickle Hb (not present in study pop<sup>n</sup>)

**Misidentified variant** excl from Total area

# D-10 HbA1c chromatograms

A. Well-determined

B. Mis-identified Hb variant

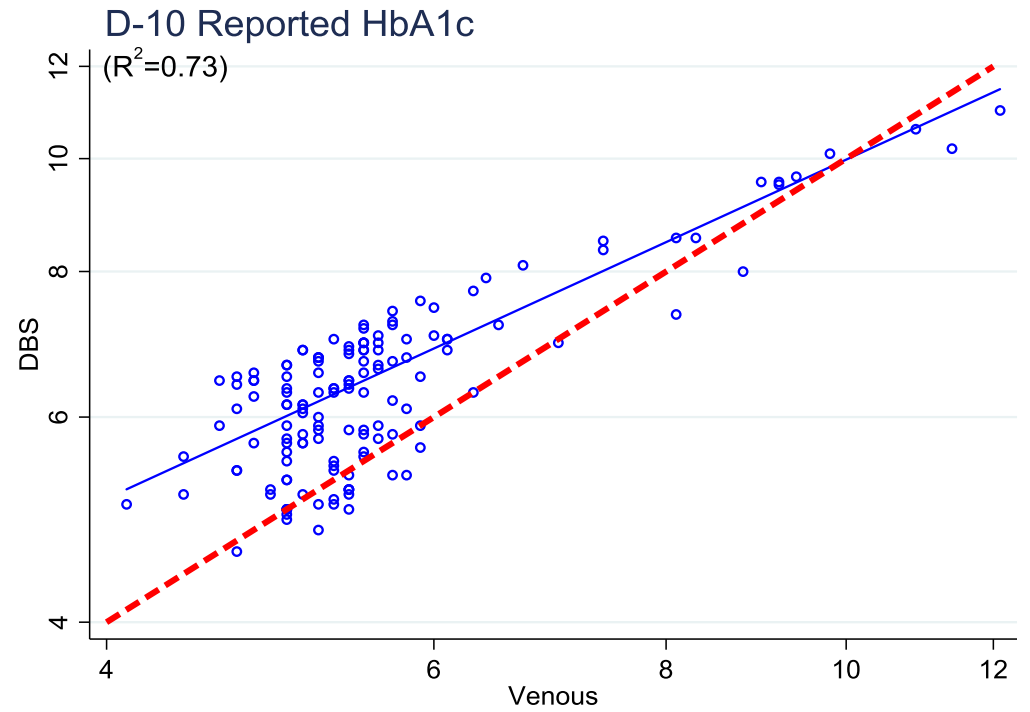


$$\% \text{HbA}_{1c} = \frac{\text{Area under } A_{1c}}{\text{Total Area incl } A_0}$$

**Variant** excluded from Total area  
⇒ D-10 reported % HbA<sub>1c</sub> ↑ biased

D-10 reported % HbA1c

Read chromatograms, add back  
**mis-identified variant** to total area

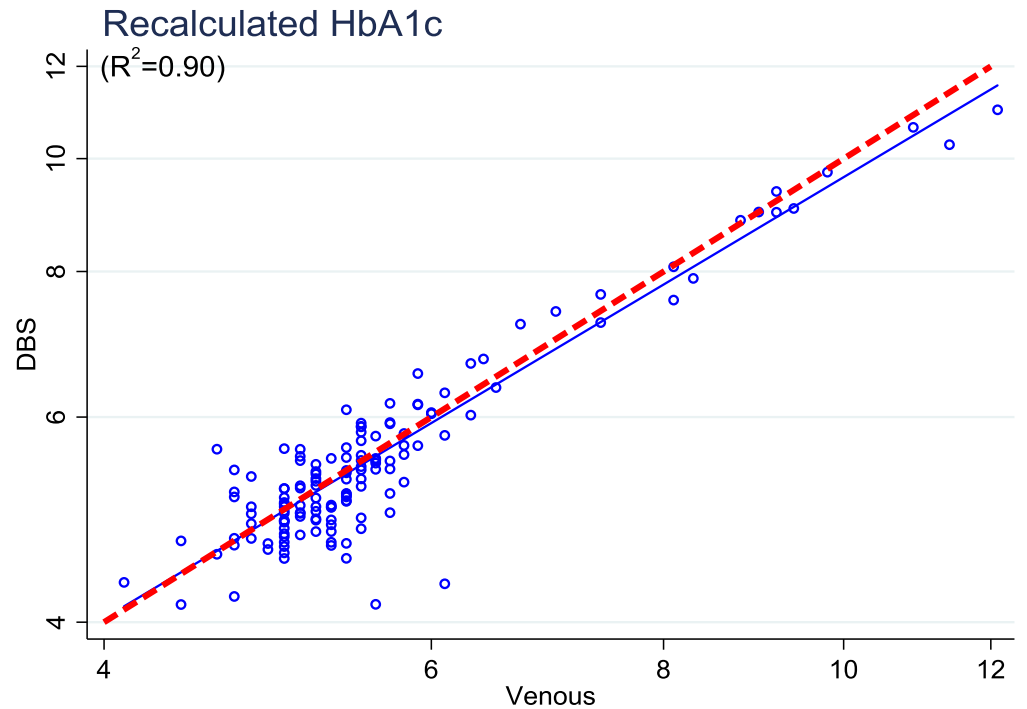
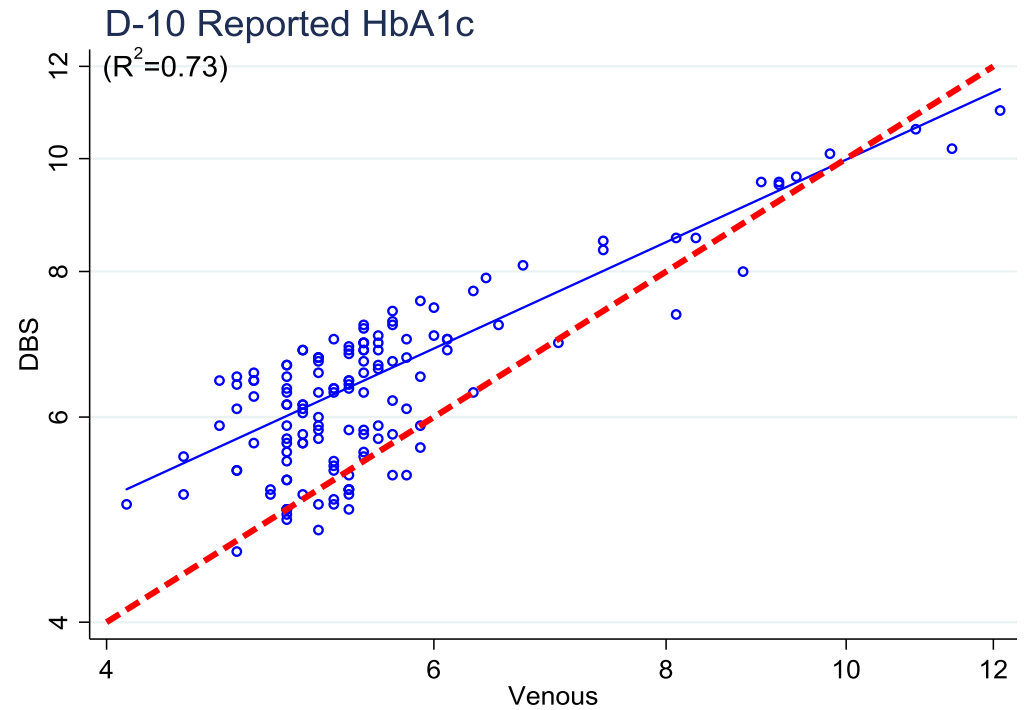


D-10 reported % HbA1c

Read chromatograms, add back  
**mis-identified variant** to total area

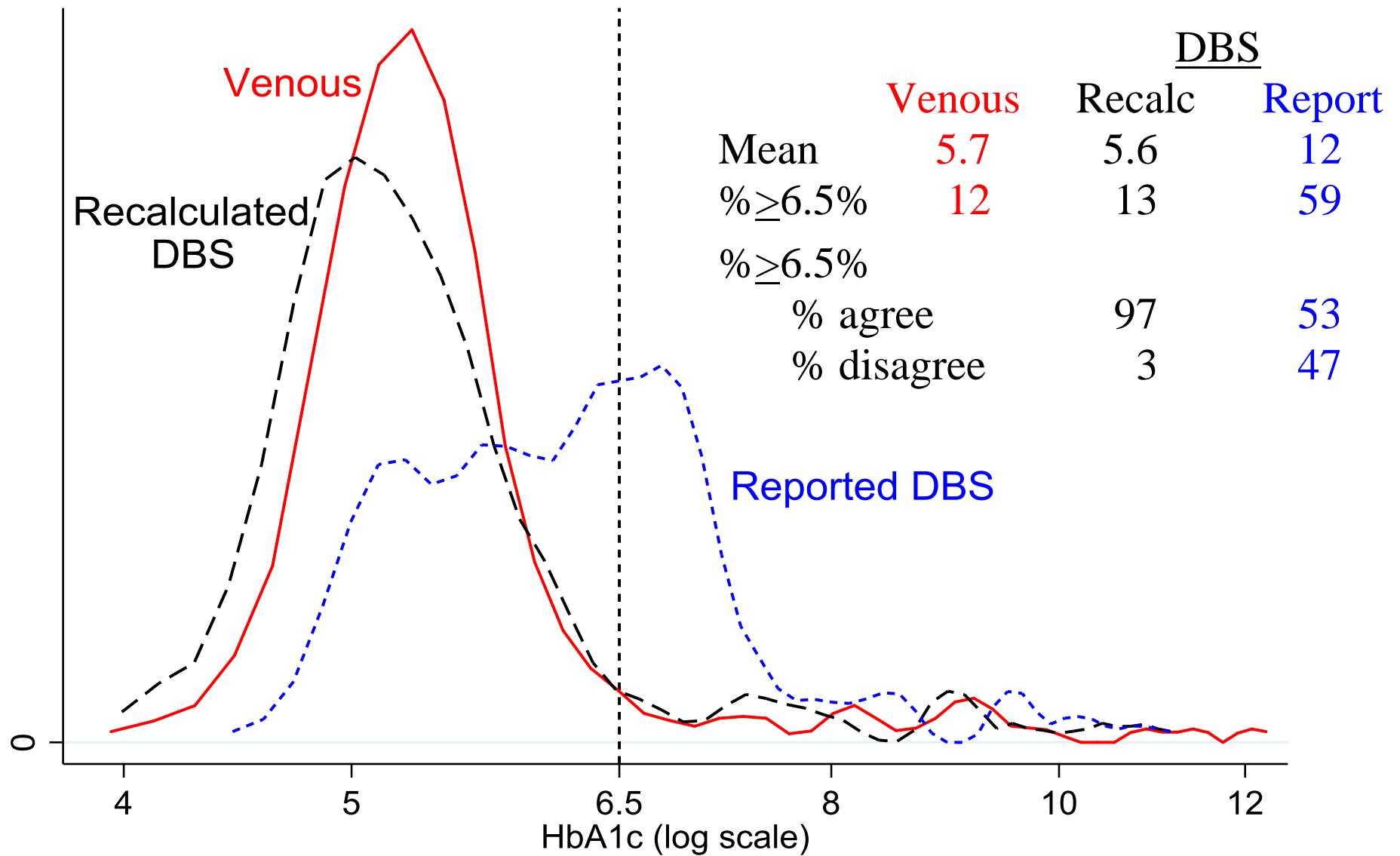
**Recalculate % HbA1c**

Recalculated %HbA1c **unbiased**



How different are these  
HbA1c distributions?

# HbA1c distributions: Venous, reported & recalculated DBS



Recalculated %HbA1c matches gold standard **very well**

DBS collected under  
'controlled' and 'field' conditions



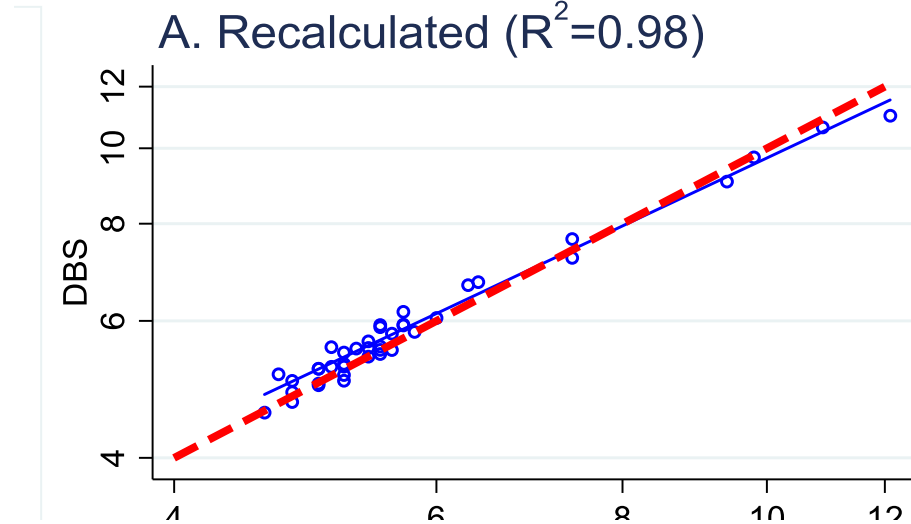
## A. 'Controlled' setting

'Control' temperature and humidity

Clinic-like

Recalculated DBS unbiased

Very close to gold standard



## A. 'Controlled' setting

'Control' temperature and humidity

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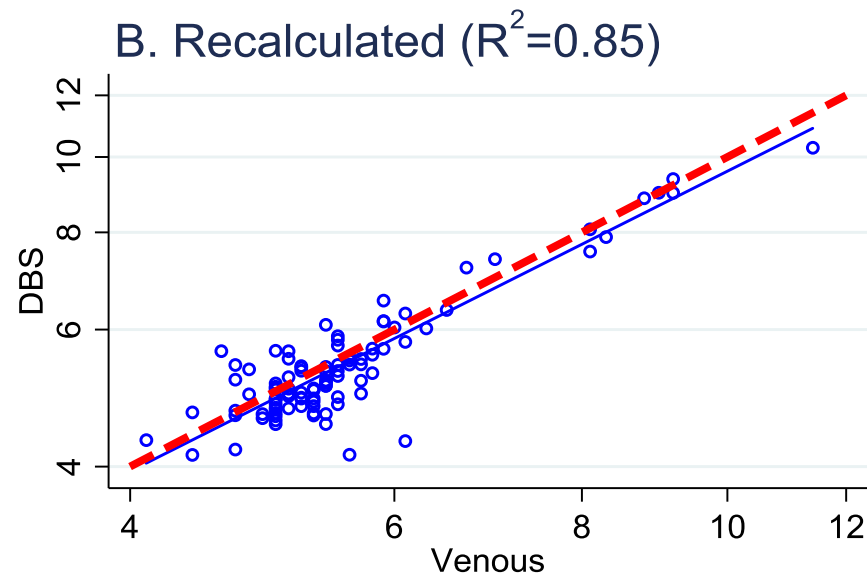
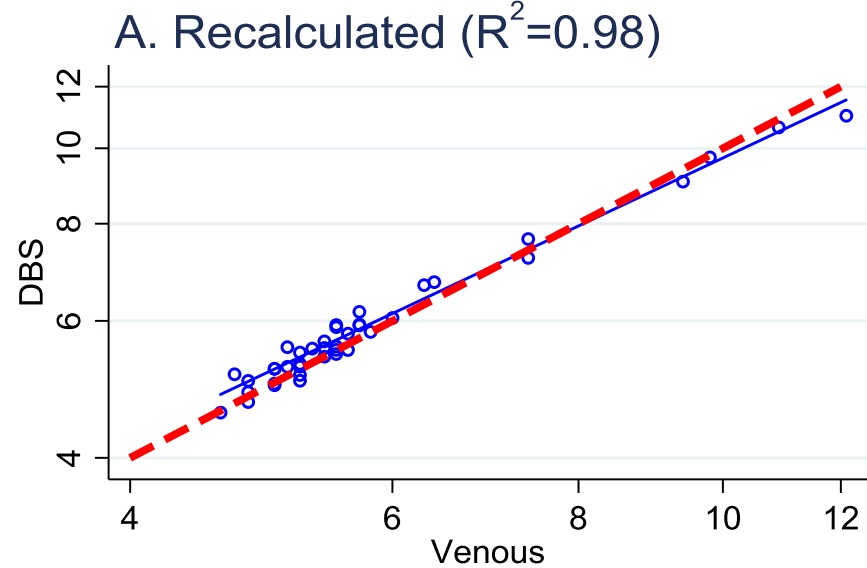
## B. 'Field' setting

Not control temp & humidity

In community center

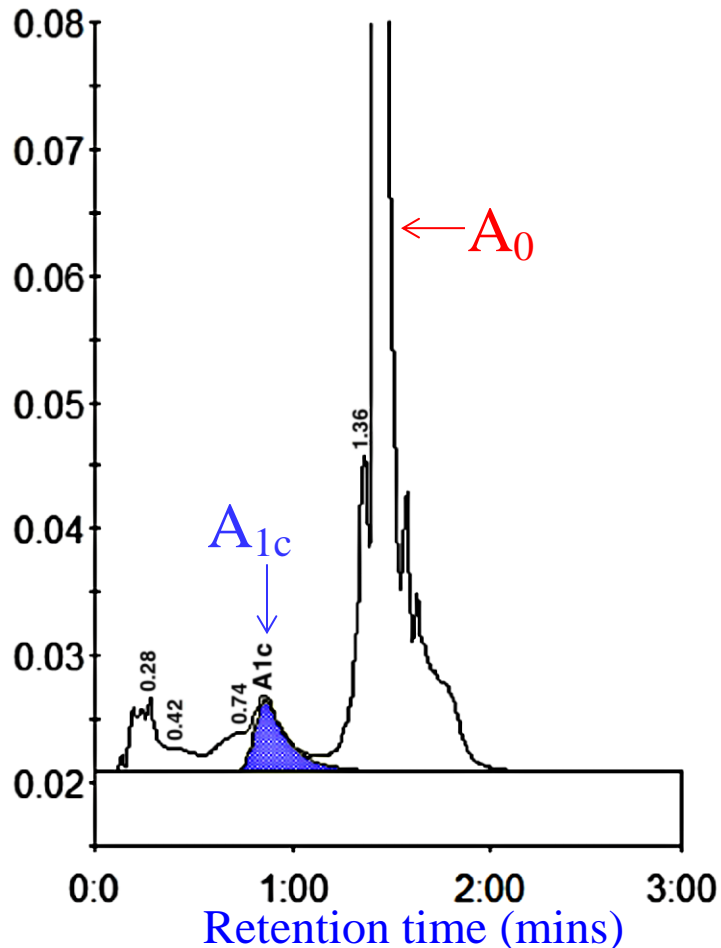
(Not as adverse as in home)

Recalculated DBS unbiased,  
noisier than controlled setting



# D-10 HbA1c chromatograms

## A. Well-determined



For DBS under ‘field’ conditions use chromatogram characteristics to separate

**Standard DBS (52%)**

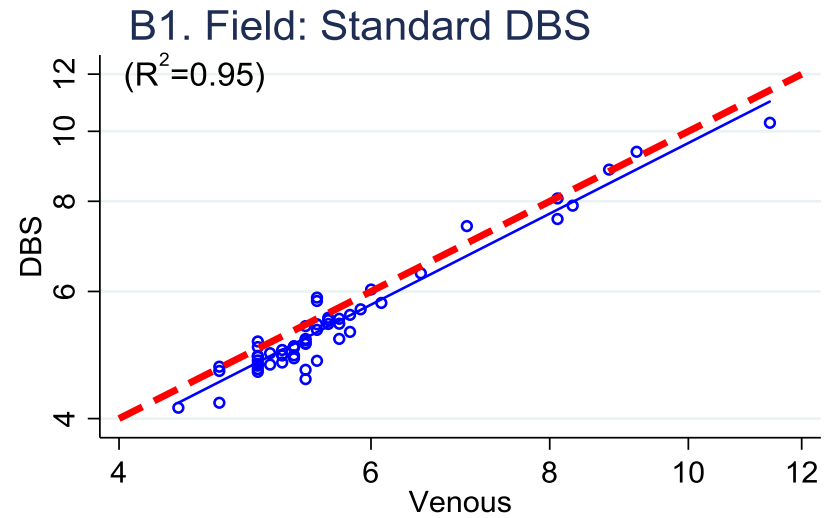
**Sub-standard DBS (48%)**

**Key distinction: Retention time for A1c**  
(Limits from BioRad D-10 doc and our ‘controlled’ setting DBS results)

# Separating standard and sub-standard field DBS

Field: Standard DBS

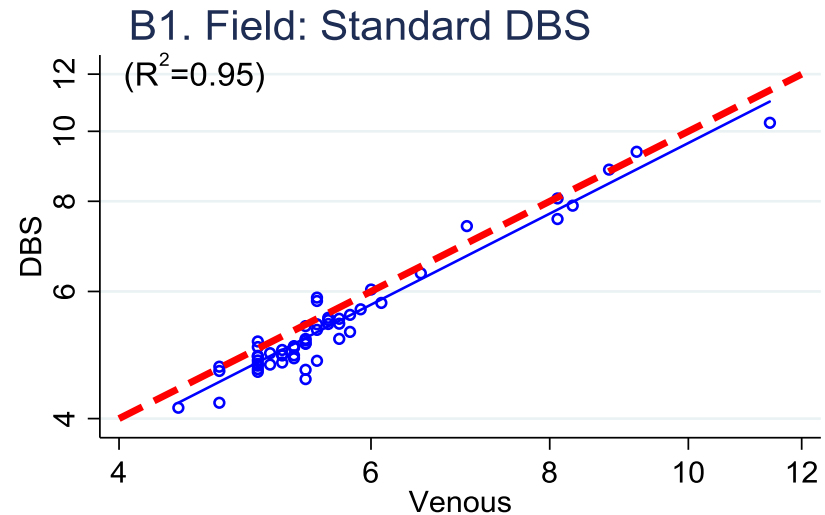
Matches gold standard well



# Separating standard and sub-standard field DBS

## Field: Standard DBS

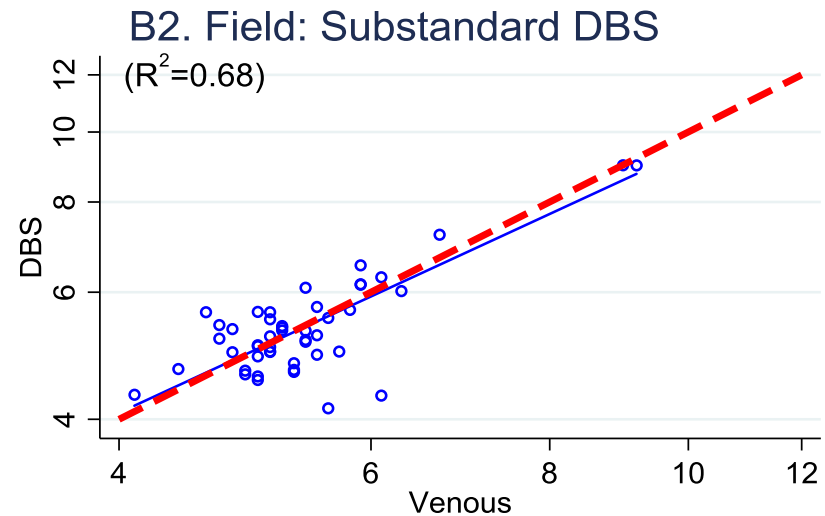
Matches gold standard well



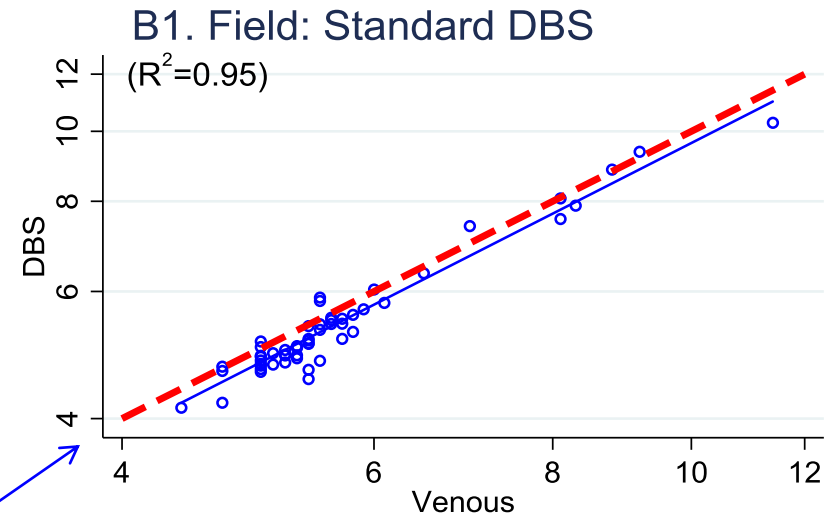
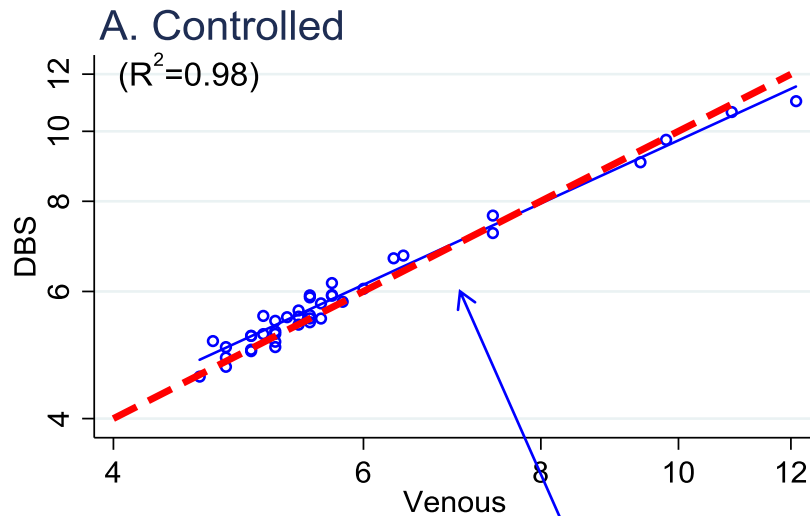
## Field: Substandard DBS

Unbiased

But very noisy (low  $R^2$ )

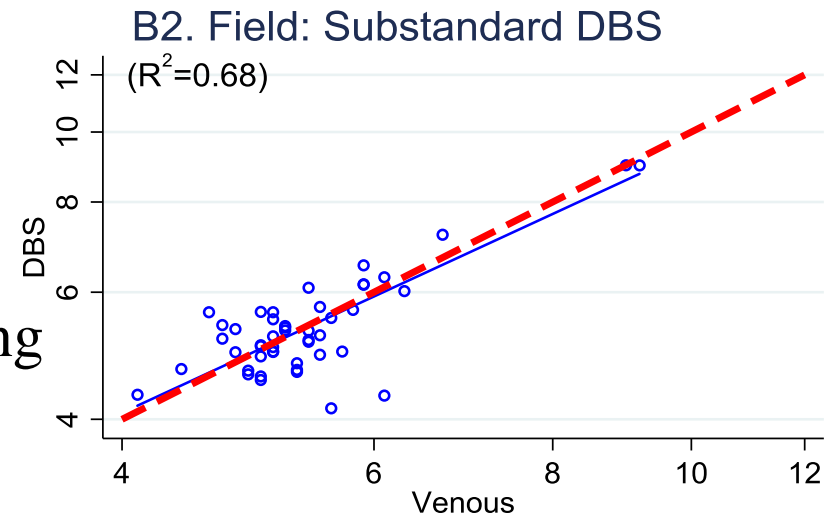


# Controlled, standard and sub-standard field DBS



Very similar results

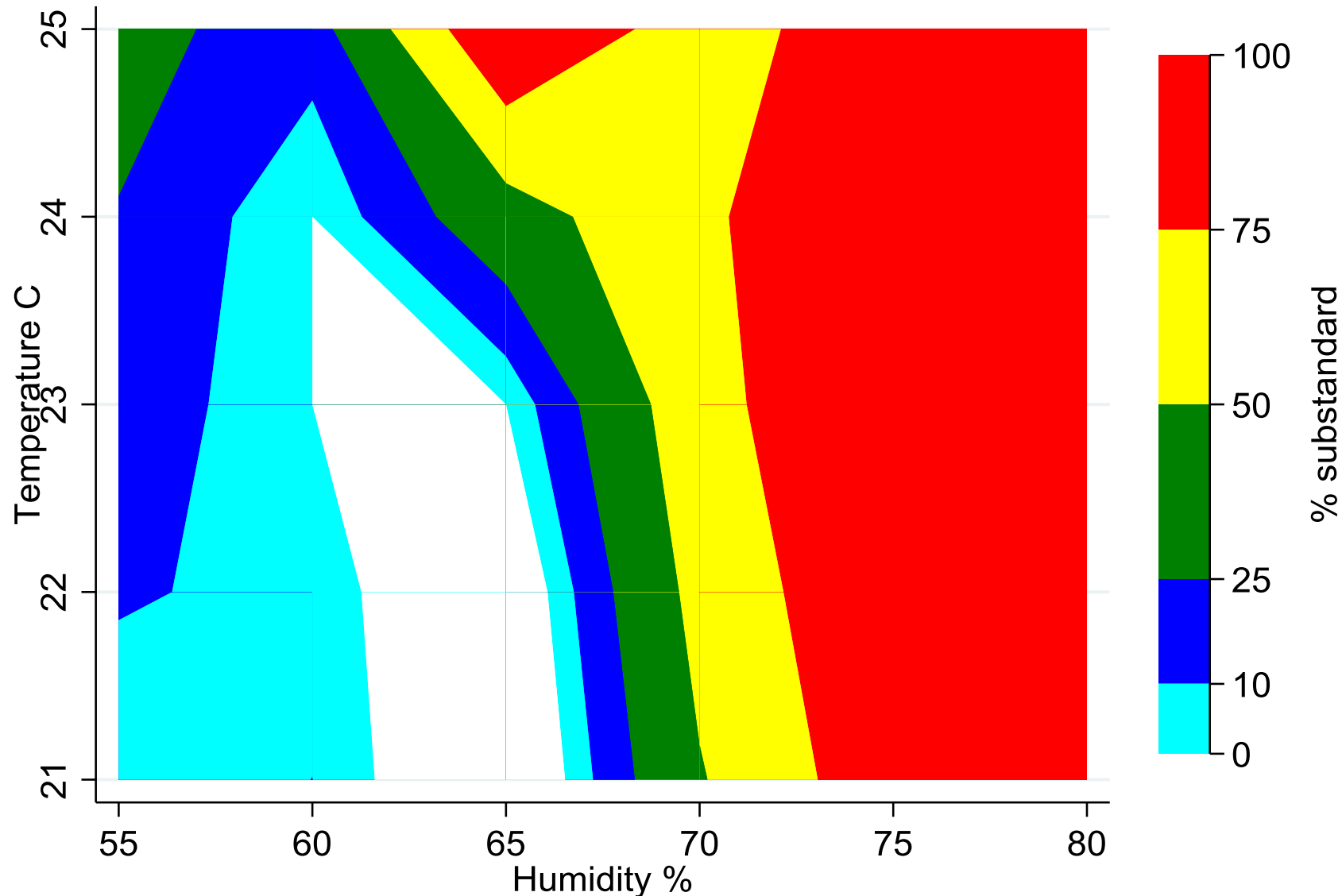
Controlled DBS (like clinic)  
Standard Field DBS



Identify Substandard Field DBS using chromatograms if noise a concern

What predicts whether 'field' DBS  
standard or substandard?

## % substandard DBS by humidity and temperature



**Suggests humidity important (>70% bad).**

But study not designed to measure effects humidity, temperature, other factors  
And only small number DBS



Conclude

# Conclusions

- %HbA1c from DBS reported by HPLC **upward biased** if DBS collected under adverse conditions
- Use chromatogram data, **recalculated** %HbA1c **unbiased**
- DBS **perform well** if collected in
  - Controlled** setting
  - Field** setting & DBS **Standard** (based on chromatograms)

But **Field** setting & **Sub-standard** DBS results **very noisy**
- Value of **test field protocols** mitigate effects temp, humidity & other potential factors
- Value of **field-based validation against gold standard** under **same field conditions as study**  
(Regular re-validation during fieldwork)