When Accuracy Alone Is Not Sufficient:

New Roles for Clinical Chemists

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Southeast Section AACC
March 26, 2014

Roles for Clinical Chemists

- Traditional Roles
  - Choose (and validate) instruments/methods
  - Generate accurate, timely results
  - Follow doctors' "orders"
  - Plus potentially others

Roles for Clinical Chemists

- Traditional Roles
- New Roles:
  - ***Help implement clinical guidelines***
    - HCV Ab testing for all Baby Boomers
    - Will detect 75% of undiagnosed cases (3M in US)
    - HCV (unlike HBV and HIV) is a <u>curable</u> disease
  - ***Explain potentially confusing tests***
    - opiate immunoassay does not detect oxycodone (or methadone)
    - with every result – not just if you're called
  - ***Help explain costs and TAT***
    - HBV viral load unnecessary for diagnosis of HBV
    - it's roughly 20 to 50-fold more expensive than HBsAg

3

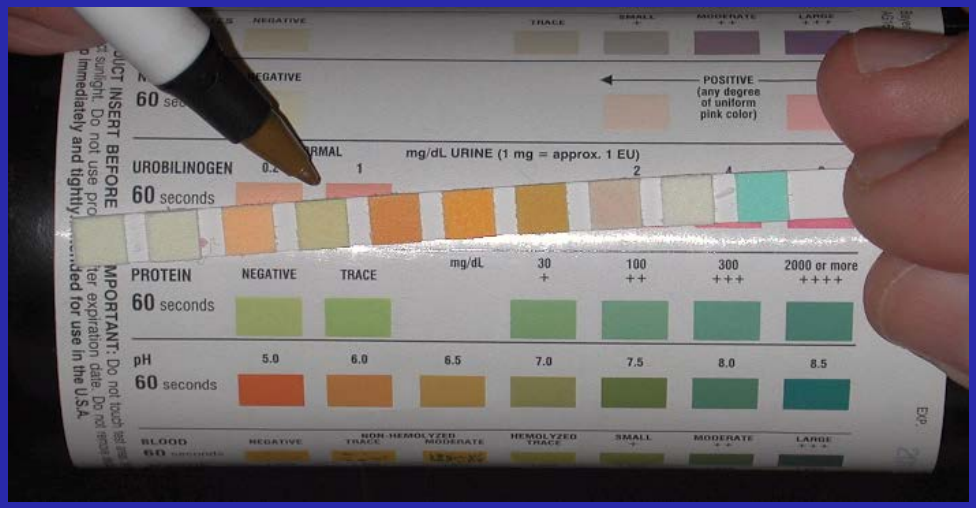
Numerous Other Examples

- Immunoassays for testosterone
  - adequate for screening adult males for hypogonadism
- Vitamin D (25 OH Vitamin D)
  - does your assay detect D_2 at 100%?
  - eliminating orders for 1,25 Dihydroxy Vitamin D
- Ethylene glycol or methanol: OK to test, but treat presumptively
- "4th Generation HIV Ab": Is it really helpful?
- Immunosuppressants (CsA, Tacro, Rapa):
  - do your physicians know results are method-dependent?
  - it may be a "Send-Out", *but you can (should?) help*
- ESR: why are most of our labs still offering it?
  - if may not be done in your lab, *but you can (should?) help*
- Glucose meter accuracy:
  - does it allow for tight (or even semi-tight) glycemic control?
  - do critical values need to be confirmed by central lab?
  - it may be not under your jurisdiction, *but you can (should?) help*

Three Examples Today
All Illustrate Why Clinical Chemists Are In a Strategic Position to Improve Care

- **Urine Dipstick Protein**
  - a genuinely awful test: we need to eliminate it
  - or, at a minimum, highlight its deficiencies
- **Urine Albumin**
  - Extremely underutilized test
  - Screening for CKD, an epidemic
- **Hemoglobin A1c**
  - more complicated than you'd think
  - a poor surrogate for fingerstick glucose
  - time permitting, we may mention fructosamine and glycated albumin

Dipstick Urine Protein



- among most common lab tests done
- lab tests: diagnostic vs screening
- discourage its use for screening
  - not simply wasteful (actually, it's very inexpensive)
  - rather, potentially misleading

Case Scenario

Dr. Jones screens all his diabetic patients by sending urine samples to your lab for dipstick proteins.

As long as the dipstick is reported as negative, he is reassured that he has ruled out early diabetic nephropathy.

Sounds reasonable, doesn't it?

Proteinuria Physiology

- virtually all proteins are too large to be filtered through a healthy glomerulus
- once proteins do leak, there is no mechanism to reabsorb them
- urine protein concentration reflects amount leaked ***plus water content of urine*** , which varies with hydration
- provides rationale for reporting urine protein not simply as concentration but as 24^{o} collection

Protein/Creatinine Ratio

- creatinine filtered through glomerulus
- largely unsecreted and unreabsorbed by tubules
- thus, its urine concentration reflects amount filtered
  plus water content of urine, which varies with hydration
- if you divide $[protein]_{urine}$ by $[creatinine]_{urine}$,
  since water content of urine is in denominator of both,
  you eliminate the effect of hydration status
- urine protein/creatinine ratio is an
  excellent surrogate for 24^{o} urinary protein
  and can be done on any spot/random urine!

$[protein]_u$ is misleading

- what can happen when you rely on [protein] alone
- NB: conventional chemistry assay is no better !!

sample	dipstick protein	(estimated) dipstick mg/dL	Chemistry protein mg/dL	creatinine mg/dL	prot/ creat ratio	
1	1+	30	38			
2	1+	30	46			
3	2+	100	86			
4	3+	300	279			
5	3+	300	358			

$[protein]_u$ is misleading

- what can happen when you rely on [protein] alone
- NB: conventional chemistry assay is no better !!

sample	dipstick protein	(estimated) dipstick mg/dL	Chemistry protein mg/dL	creatinine mg/dL	prot/ creat ratio	
1	1+	30	38	47	0.8	
2	1+	30	46	352	0.1	
3	2+	100	86	55	1.6	
4	3+	300	279	137	2.0	
5	3+	300	358	230	1.6	

False Negative Type 1

- **Bence-Jones Protein (BJP)**
  - **monoclonal free light chains**
  - **by definition, very small (23 kD)**
  - **so small, filtered by normal glomerulus (even without albuminuria!!)**

- ***not*** **detected by dipstick method**

Adapted from
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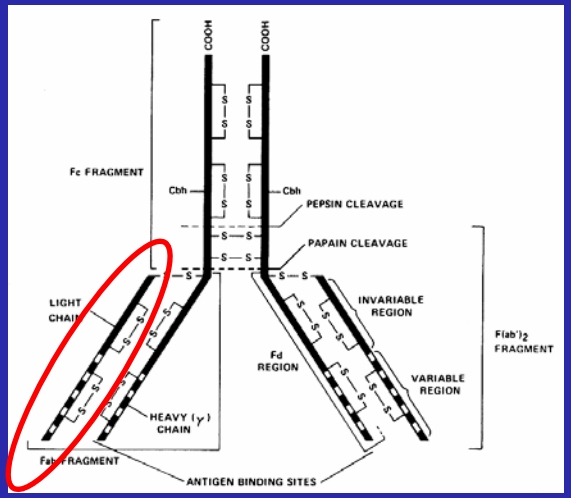


Urine Protein Methods

- **dipstick:**
  - **method: protein error of pH indicators (c1909)**
  - **detects albumin > globulin > BJP**

- **conventional chemistry assay:**
  - **method: denature protein, then detect resulting turbidity using spectrophometry**
  - **sensitive to all proteins, including BJP**

➔ **If a sample is dipstick negative, chemistry positive, it's probably BJP**

- **(micro)albumin:**
  - **method: immunoassay**
  - **detects *only* albumin**

False Negative Type 2

- dipstick protein is not sensitive enough
  to rule out pathologic levels of proteinuria
  cannot distinguish low levels from 0

- definition:
  - analytic sensitivity = how low you can go?

- assays for which sensitivity is particularly important:
  - TSH (3rd generation)
  - CRP ("hs-CRP")
  - Troponin
  - D-Dimer
  - *and, yes, urine protein!*

Analytic Sensitivities

method	value from package insert	value in (mg/dL)	sensitivity (relative to dipstick)
Dipstick	18 mg/dL	18	
Urine Protein	6 mg/dL	6	3X more sensitive
Urine Albumin (microalbumin)	3 mg/L	0.3	>50X more sensitive
Serum Protein	0.2 g/dL	200	
Serum Albumin	0.1 g/dL	100	

Is Sensitivity Needed?

- pathologic proteinuria defined as
  - 30 mg protein/g creatinine for diabetics
  - 300 mg protein/g creatinine for others
- typical range of spot urine creatinine: 20-200 mg/dL

method	sensitivity	dilute $[creatinine]_u = 20$	concentrated $[creatinine]_u = 200$	
dipstick	18 mg/dL	~~900 mg/g~~	~~90 mg/g~~	X
conventional chemistry	6 mg/dL	~~300 mg/g~~	30 mg/g	?
urine albumin	0.3 mg/dL	15 mg/g	1.5 mg/g	√

Summary: Take Home Points

- **Limitations of urine dipstick protein assay:**
  - **without creatinine, quantitation can be misleading**
  - **a negative does not rule out BJP**
  - **a negative does not rule out pathologic microalbuminuria**

Microalbumin Semantics

- not a different kind of albumin
  - same 60 kD protein found in serum

- rather, “micro” refers to small amounts
  - *typically mg/L*
    - serum protein is *g/dL* (10,000-fold greater)
    - urine protein is *mg/dL* (10-fold greater)

Case Scenario

Dr. Smith has been following a diabetic patient with serial urine microalbumin/creatinine ratios at your laboratory. His values have consistently been reported as less than 30 mg/g (within the normal range).

On his most recent visit, a urine dipstick protein was reported as 4+ (corresponding to >300 mg/dL), but the microalbumin/creatinine ratio on the same sample was again reported as less than 30 mg/g.

Dr. Jones is confused by these results, so he calls you to find out what's going on.

Microalbumin Physiology

- at 60 kD, among the smallest proteins
- leaks through glomerulus at earliest stage of disease, when larger proteins are not filtered
- makes it an excellent early indicator of disease

Microalbumin: The Numbers

- originally, 24º urine collections were advocated
  - disease threshold was 300 mg/24º
- but, 24-hour urine collections are notoriously ***difficult*** ***and*** ***inaccurate***
- so, like urine protein, current recommendation is:
  - a random/spot urine for albumin/creatinine ratio

The Numbers: Closer Look

- **remember relative sensitivities:**
  - **urine albumin *0.3 mg/dL* vs. urine protein *6 mg/dL***

- **absent a multiplier, urine albumin/creatinine ratios would be fractions**
  - **protein/creatinine: mg/mg creatinine**
  - **albumin/creatinine: mg/g creatinine (mg/mg x1000)**

- **an example will help clarify:**
  - **creatinine=50mg/dL, protein=10 mg/dL, albumin=8 mg/dL**

➔ protein/creatinine = 10/50 = 0.20

➔ albumin/creatinine = 8/50 x 1000 = 160 *(not 0.16!!)*

Who Should Be Tested?

- ***diabetics*** **should be screened** ***annually***
  - **akin to glycated hemoglobin quarterly**
  - **easy to do – requires only a spot urine**
- **diabetes: leading cause of End Stage Renal Disease**
- **evidence exists to show that early therapy can:**
  - **slow progression of diabetic kidney disease**
  - ***perhaps even reverse it!***
- **only 10% of Medicare diabetic patients get screened**

Not Just for Diabetics

- **End Stage Renal Disease**
  - **affects** ***99,000*** ***people in US***
    - **more than number of breast & colon cancer deaths combined**
    - **costs $20 billion per year, more than the entire NIH budget**
- **Chronic Kidney Disease:**
  - **affects** ***20,000,000*** ***people in US***
    - **8,000,000 with decreased GFR**
    - **12,000,000 with proteinuria**

NKF Recommendations

(www.nkdep.nih.gov)

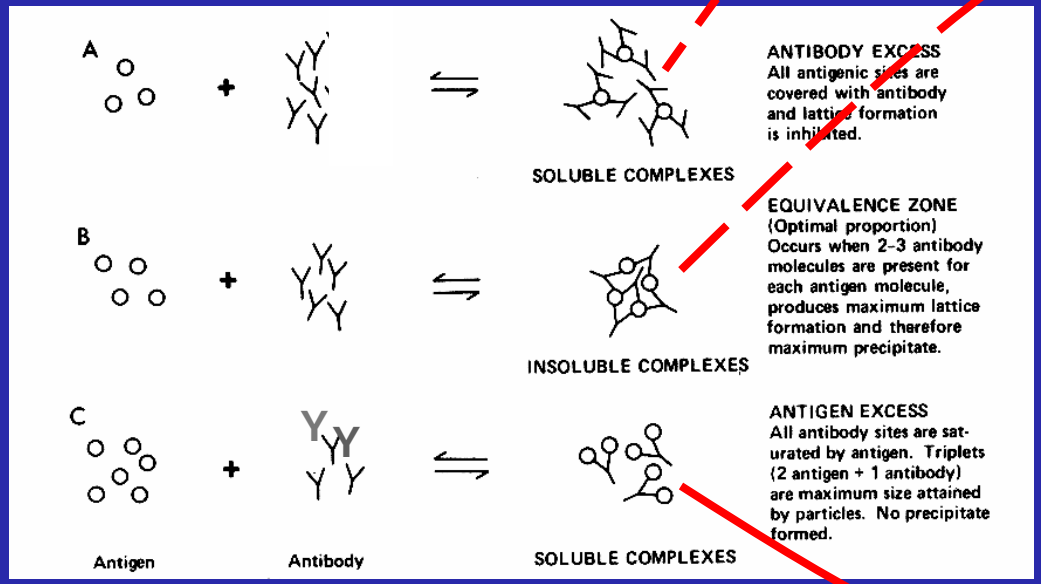
- **Screen high risk groups as follows**
  - ***serum creatinine***
    - **lab report should include *estimated GFR* (by MDRD equation) (*serum creatinine should not be your final answer . .)***
  - ***urine albumin/creatinine on random spot urine***

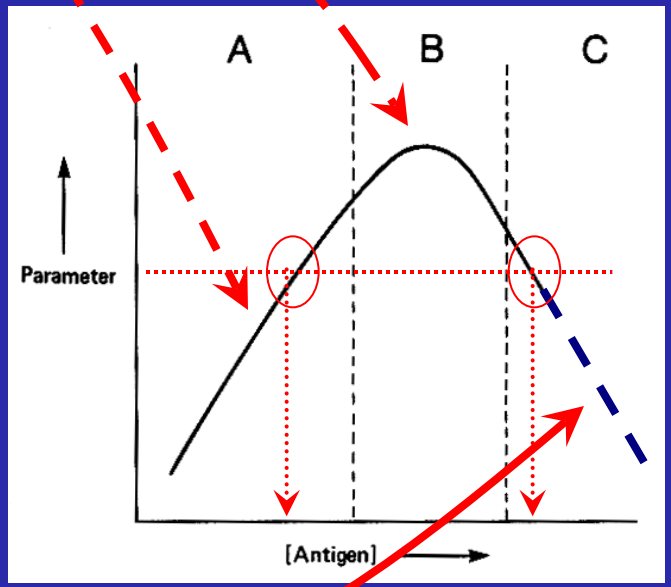
- **High Risk Groups include patients**
  - **with diabetes mellitus**
  - **with hypertension**
  - **with family history of kidney disease**
  - **who have taken analgesics in the past year**

Let's Get the Right Answer!

- **urine albumin tests are extremely sensitive**
  - **because they are done by *immunoassay***
- **typically, homogeneous immunoassay methods (i.e., no separation step)**
  - **subject to *"hook effects"***
- **if you're not very careful,**
  - **you can get *falsely low* (*even negative*) *results***
  - **with *no error messages!***

Hook Effect: What Is It?





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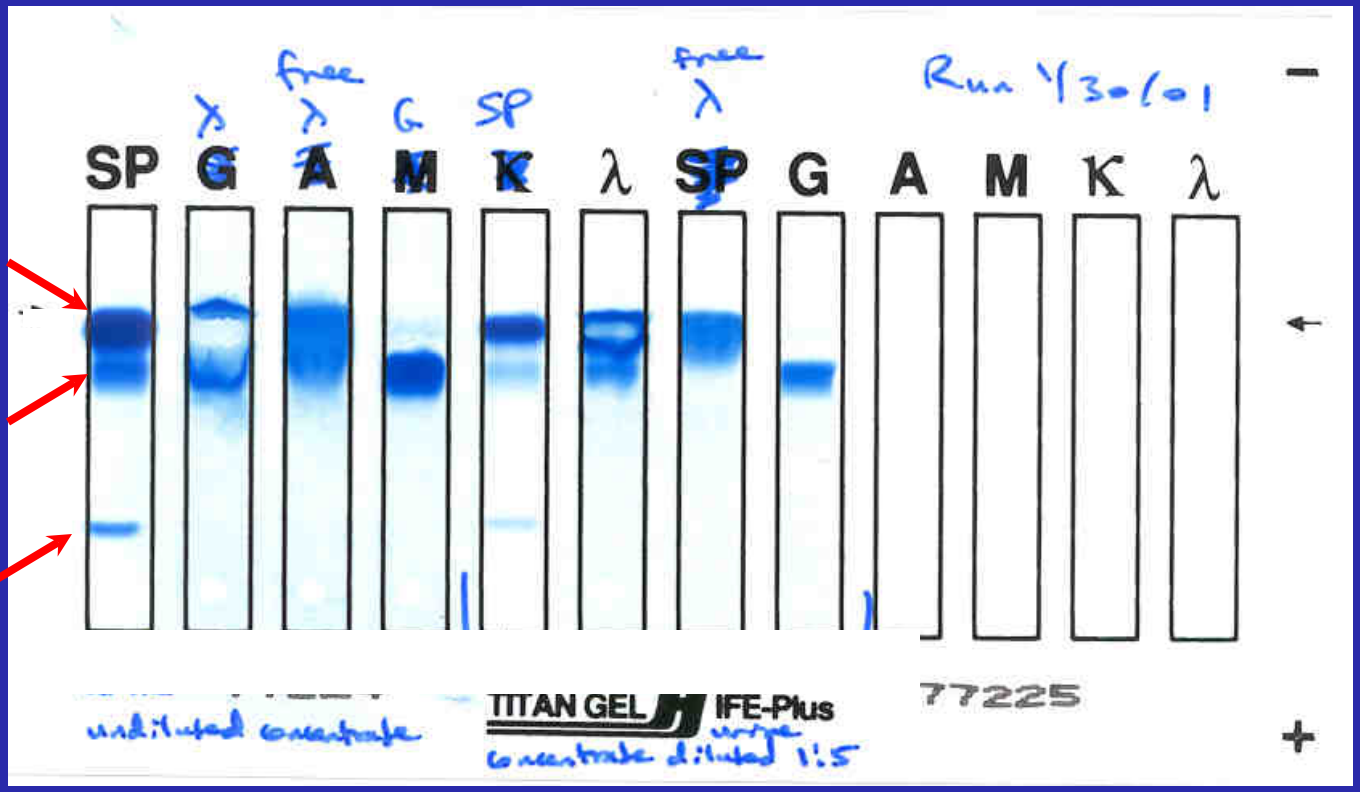
Hook Effect: A Picture

free λ

intact IgG λ

albumin



Prevalence & Prevention

- example: Roche/Hitachi users
  - disclaimer is in package insert
  - among visitors to BIDMC,
    - few knew of it, and
    - fewer were taking steps to account for it!

Albumin

Tina-quant ® Albumin

Roche

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Calibration

Traceability: This method has been standardized against CRM 470.

Roche/Hitachi 902 analyzers

S1	0.9% NaCl
S2-6	C.f.a.s. PUC

Calibration is performed with C.f.a.s. PUC via serial dilution (6-point calibration).
Preparation of S1-6:

No.	NaCl solution (0.9%)	C.f.a.s. PUC	Assigned value conversion factor
1	200 µL	–	0.0
2	1440 µL	20 µL	0.01370
3	860 µL	20 µL	0.02273
4	420 µL	20 µL	0.04545
5	110 µL	100 µL	0.47619
6	–	200 µL	1.00000

The calculated values for the dilution series are keyed into the analyzer.

Roche/Hitachi 917/MODULAR analyzers

S1	0.9% NaCl
S2-6	C.f.a.s. PUC

Calibration is performed with C.f.a.s. PUC via serial dilution made

Calculation

The analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mg/L x 0.0152 = µmol/L

Limitations - interference[7]

Criterion: Recovery within ± 10% of initial value.

Icterus: No significant interference up to an approximate conjugated bilirubin concentration of 66 mg/dL or 1128 µmol/L.
Hemolysis: No significant interference up to an approximate hemoglobin concentration of 300 mg/dL or 186 µmol/L.
No interference by acetone < 60 mmol/L, ascorbic acid < 5.68 mmol/L, creatinine < 44.2 mmol/L, glucose < 111 mmol/L, uric acid < 4.17 mmol/L, urea < 700 mmol/L and urobilinogen < 338 mmol/L.
Seventeen frequently used pharmaceuticals were tested in vitro. No interference with the assay was found.
With the exception of the MODULAR P antigen excess check application, a high-dose hook effect may occur at albumin concentrations above 2500 mg/L (38.0 µmol/L).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

Roche/Hitachi 902 analyzers

Measuring range:** 3–400 mg/L (0.046–6.08 µmol/L)
At higher concentrations manually dilute the sample with 0.9% NaCl (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

Roche/Hitachi 911/912/917/MODULAR analyzers

Measuring range:** 3–400 mg/L (0.046–6.08 µmol/L)
Extended measuring range with rerun:*** 3–3000 mg/L (0.046–45.6 µmol/L)

To eliminate the possibility of reporting falsely low results on specimens in excess of the Heidelberger limit (2500 mg/L), test these specimens with a urine dipstick and dilute appropriately before performing the assay. Multiply the result obtained by the appropriate dilution factor.

Prevalence & Prevention

- example: Roche/Hitachi users
  - disclaimer is in package insert
  - among visitors to BIDMC,
    - few knew of it, and
    - fewer were taking steps to account for it!

- prevention strategies:
  - compare total protein and albumin
  - run every urine sample neat and on dilution
  - dipstick protein to the rescue!
    - not an immunoassay
    - not subject to hook effects

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Summary: Take Home Points

- **Limitations of urine dipstick protein assay:**
  - **without creatinine, quantitation can be misleading**
  - **a negative does not rule out BJP**
  - **a negative does not rule out pathologic microalbuminuria**

- **For CKD proteinuria screening, including diabetes,**
  - **make sure correct test is ordered (urine albumin/creatinine)**
  - **make sure you get the correct answer**
    - **rule out "hook effect" – dilution, total protein, dipstick protein**

Hemoglobin A1c

- A great, but far from perfect, test

Case Scenario

Mr. Donaldson, a 62-year old man with Hemoglobin SC disease, was diagnosed with diabetes mellitus following two elevated fasting blood sugars. He was placed on a diet and instructed on the use of a glucose meter to monitor his glucose levels at home.

As noted below, his first hemoglobin A1c was reported as 5.4%. Then, following a change in methodology, it was reported several times over the next 18 months as 6.4 - 7.3%. Following another change in methodology, the value was reported as 4.8%; at the same time, a fasting glucose was 138 mg/dL.

Does Mr. Donaldson really have diabetes?
Why is his A1c so dependent on the methodology used?

	Nov 02	Dec 02	Feb 03	Jul 03	Oct 03	Jun 04	Jul 05
fasting glucose	152	154					123
A1c (Hitachi)		5.4%					4.8%
A1c (Integra)			7.3%	6.8%	6.6%	6.4%	6.6%
A1c (Tosoh)			error	error	error	error	error "1.9%"

Diagnosis of Diabetes

- any of the following, on 2 separate occasions:
  - fasting glucose > 126 mg/dL
  - random glucose > 200 mg/dL
    accompanied by symptoms of hyperglycemia
  - 2-hour glucose > 200 mg/dL
    following a 75g oral glucose load
  - Hemoglobin A1c > 6.5%

Gray Top Tubes

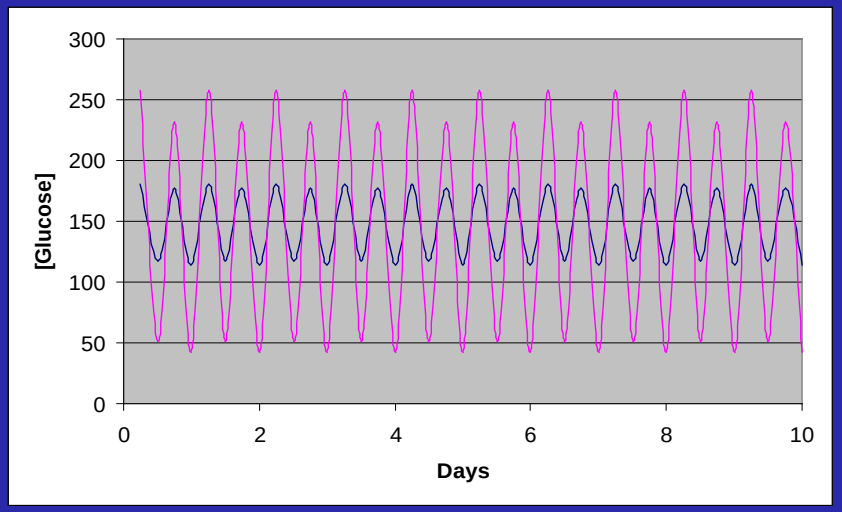
- glucose in whole blood
  - decreases by 7 mg/dL/hour at room temperature (RT)
  - secondary to the metabolism of RBCs
- effect is not trivial
  - [glucose] depressed 28 mg/dL after just 4 hours at RT
- prevention
  - centrifugation: separate the serum/plasma from RBCs
  - refrigeration: metabolism is slowed at low temperature
  - gray top tube: fluoride inhibits glycolysis

A Few Clinical Points About A1c

- A_1c is mean blood glucose (BG) over time
  fingerstick (spot) glucoses are just as important
- process is non-enzymatic: glycation, not glycosylation
- knowing the conversion (A_1c → mean BG) is helpful [eAG]
- be aware of standardization: IFCC vs NGSP/DCCT
- know current (NGSP/DCCT) guidelines: 6.5%, <7.0%, >8.0%
- [A_1c] varies not only with glucose but also with RBC-lifespan

Mean vs. Spot [Glucose]

- one can achieve a 7% A_1c many different ways:



- goal is not only to lower A_1c
  but also to smooth excursions around the mean
- one needs Fingerstick Glucoses as well as A_1c

Glycation

- non-enzymatic process – avoid the term “glycosylation”
- occurs at N-terminal end (=valine) of beta chain
- if there are no beta chains (e.g., Hb F),
  there’s no glycation at the N-terminus
- Hb S and Hb C involve amino acid substitutions
  at position 6 (=valine), just 5 amino acids away

Converting A_1c to Mean BG

- need to remember just 2 facts:
  - in healthy individuals, mean BG=100 and [A_1c]=5% (roughly)
  - for each 1% increase in A1c, 30 mg/dL increase in mean BG
- so, an 8% [A_1c] corresponds to a 190 mean BG
  - 100 + [(8%-5%)x30] = 100 + (3x30) = 190 (my equation)
- *versus official equation*
  - ~~*MBG = (35.6 x HbA1c) – 77.3 = 35.6 x 8 – 77.3 = 207*~~

~~Diabetes Care 2002;25:275-278~~

Diabetes Care 2008; 31:1-6
(August, 2008)

Translating the A1C Assay Into Estimated Average Glucose Values

David M. Nathan, MD[1]
Judith Kuenen, MD[2]
Rikke Borg, MD[3]
Hui Zheng, PHD[1,4]
David Schoenfeld, PHD[1,4]
Robert J. Heine, MD[2]
for the A1c-Derived Average Glucose (ADAG) Study Group*

National Glycohemoglobin Standardization Program values (13), potentially causing confusion for patients and health care providers. Moreover, the International Federation of Clinical Chemists re-

RESEARCH DESIGN AND METHODS — A total of 507 subjects, including 268 patients with type 1 diabetes, 159 with type 2 diabetes, and 80 nondiabetic subjects from 10 international centers, was included in the analyses. A1C levels obtained at the end of 3 months and measured in a central laboratory were compared with the AG levels during the previous 3 months. AG was calculated by combining weighted results from at least 2 days of continuous glucose monitoring performed four times, with seven-point daily self-monitoring of capillary (fingerstick) glucose performed at least 3 days per week.

RESULTS — Approximately 2,700 glucose values were obtained by each subject during 3 months. Linear regression analysis between the A1C and AG values provided the tightest correlations ($AG_{mg/dl} = 28.7 \times A1C - 46.7$, $R^2 = 0.84$, $P < 0.0001$), allowing calculation of an estimated average glucose (eAG) for A1C values. The linear regression equations did not differ significantly across subgroups based on age, sex, diabetes type, race/ethnicity, or smoking status.

CONCLUSIONS —

IFCC Standardization
(not yet implemented, at least in US)

- for healthy non-diabetic individuals,
  the reference (normal) range is

- NGSP/DCCT: 4.8% - 5.9%
- IFCC: 2.9% - 4.2%

- in other words, [A_1c] will decrease by
  an absolute 2% (or roughly 40%)!

- Rest of the world:

IFCC (mmol/mol) = (DCCT (%) – 2.15) * 10.929
48 mmol/mol 6.5%

Current Reference Ranges
(NGSP/DCCT standardization)

- **healthy individuals (Roche): 4.8% - 5.9%**
- **diabetics (American Diabetes Association):**

  <7.0% = goal of therapy

  >8.0% = warrants therapeutic action

Things Other Than Glucose Affect A_1c

- reference ranges assume normal RBC lifespan
  - with hemolytic anemias,
    RBCs are not around as long,
    and A_1c is not as high as one would expect
  - similarly, with transfusion,
    one is adding blood whose A_1c value is
    unrelated to the recipient's mean BG

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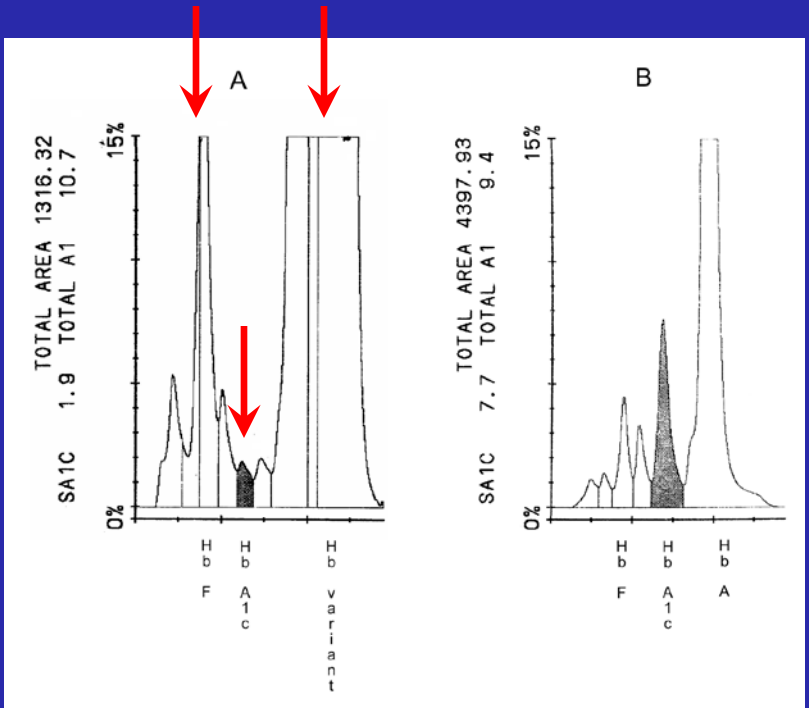
Chromatogram
(Tosoh 2.2 Plus)

Mr. Donaldson



Typical Patient

BIDMC data

Not Really "News" (I Was Just Unaware of It)

Table 1. Average differences from the comparison method for samples containing either Hb C or S traits.[a]

Method	Hb C trait		Hb S trait	
	6% Hb A_{1c}	9% Hb A_{1c}	6% Hb A_{1c}	9% Hb A_{1c}
A1c 2.2 Plus	−0.10	−0.24	−0.10	−0.54
DCA 2000	0.26	0.60	0.17	0.52
Diamat	−0.42	−0.47	0.83[b]	0.38
Diatrac	−0.39	−0.94[b]	−1.28[b]	−2.08[b]
IMx	2.87[b]	3.17[b]	0.64	0.36
Tina-quant II	−0.32	−0.44	−0.19	−0.32
Variant A1c	0.07	−0.04	0.66[b]	0.49

[a] Deming regression analysis was performed using the CLC 330 as the comparison method. The average differences of each of the other seven methods at clinical decision cutoffs of 6% and 9% were calculated for each Hb trait. To correct for intermethod calibration differences, the mean difference for homozygous Hb A samples was subtracted from tha
containing Hb C or Hb S trait.

[b] Both statistically significant (P <0.01) and clinic
>0.9% at 6% and 9% Hb A_{1c}, respectively) difference

Frank EL et al, Clin Chem 2000;46:864-867.

Table 1. Average differences from the comparison method for samples containing either Hb C or S trait.[a]

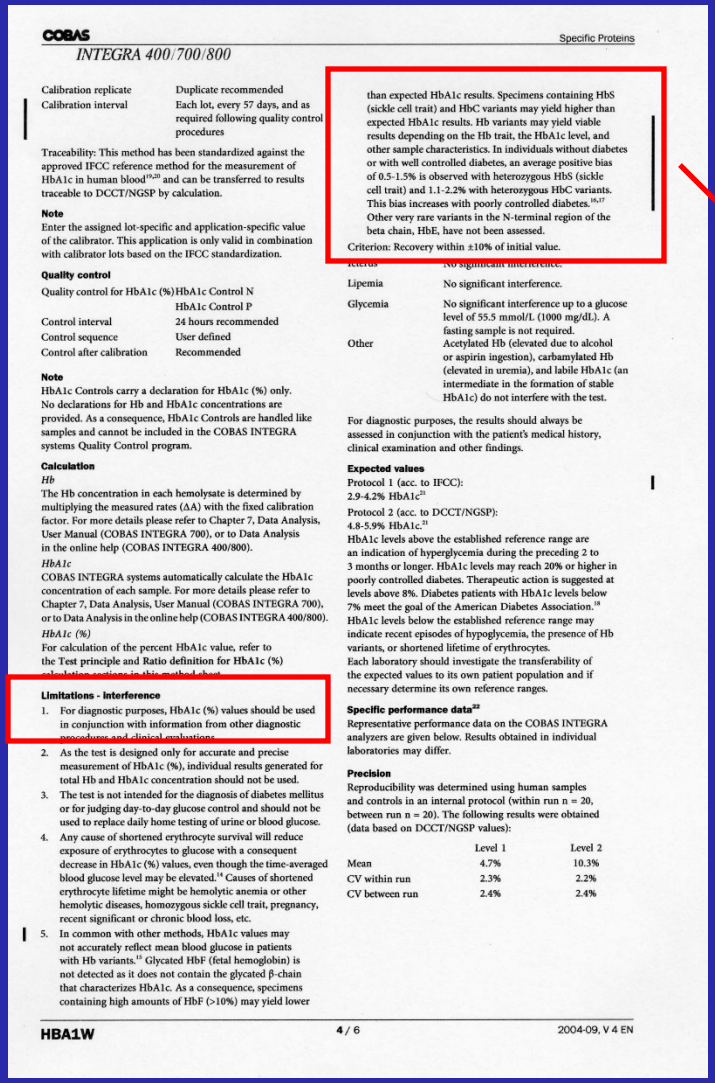
Method	Assay principle	Hb C trait		Hb S trait	
		6% Hb A_{1c}	9% Hb A_{1c}	6% Hb A_{1c}	9% Hb A_{1c}
Cobas Integra	Immunoassay	2.18[b]	4.10[b]	1.45[b]	2.74[b]
Glyco-Tek	Boronate affinity	1.37[b]	1.45[b]	0.47	0.53
Glycosal	Boronate affinity	0.63[b]	0.72	0.37	0.55
HA8140	Ion exchange	0.22	0.28	0.81[b]	0.57
Nycocard	Boronate affinity	0.23	0.07	−0.13	−0.13
Synchron CX7	Immunoassay	−0.52	−0.27	−0.41	−0.19
Variant II	Ion exchange	0.42	0.65	0.57	0.43
Variant GHb	Boronate affinity	0.59	0.86	0.40	0.66

[a] Deming regression analysis was performed using the CLC 330 as the comparison method. The average differences (%) of each of the other eight methods at clinical decision cutoffs of 6% and 9% were calculated for each Hb trait. To correct for intermethod calibration differences, the mean difference for homozygous Hb A samples was subtracted from that calculated for samples containing Hb C or Hb S trait.

[b] Clinically significant (>0.6% or >0.9% Hb A_{1c} at 6% and 9% Hb A_{1c}, respectively) differences were found.

Roberts WL et al, Clin Chem 2002;48:383-385.

Roche Acknowledges the Issue in Integra Package Insert



COBAS
INTEGRA 400/700/800

Specific Proteins

Calibration replicate	Duplicate recommended
Calibration interval	Each lot, every 57 days, and as required following quality control procedures

Traceability: This method has been standardized against the approved IFCC reference method for the measurement of HbA1c in human blood[19,20] and can be transferred to results traceable to DCCT/NGSP by calculation.

Note
Enter the assigned lot-specific and application-specific value of the calibrator. This application is only valid in combination with calibrator lots based on the IFCC standardization.

Quality control
Quality control for HbA1c (%) HbA1c Control N
HbA1c Control P

Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

Note
HbA1c Controls carry a declaration for HbA1c (%) only. No declarations for Hb and HbA1c concentrations are provided. As a consequence, HbA1c Controls are handled like samples and cannot be included in the COBAS INTEGRA systems Quality Control program.

Calculation
Hb
The Hb concentration in each hemolysate is determined by multiplying the measured rates (ΔA) with the fixed calibration factor. For more details please refer to Chapter 7, Data Analysis, User Manual (COBAS INTEGRA 700), or to Data Analysis in the online help (COBAS INTEGRA 400/800).

HbA1c
COBAS INTEGRA systems automatically calculate the HbA1c concentration of each sample. For more details please refer to Chapter 7, Data Analysis, User Manual (COBAS INTEGRA 700), or to Data Analysis in the online help (COBAS INTEGRA 400/800).

HbA1c (%)
For calculation of the percent HbA1c value, refer to the **Test principle** and **Ratio definition for HbA1c (%)**

Limitations - interference
1. For diagnostic purposes, HbA1c (%) values should be used in conjunction with information from other diagnostic procedures and clinical evaluations.
2. As the test is designed only for accurate and precise measurement of HbA1c (%), individual results generated for total Hb and HbA1c concentration should not be used.
3. The test is not intended for the diagnosis of diabetes mellitus or for judging day-to-day glucose control and should not be used to replace daily home testing of urine or blood glucose.
4. Any cause of shortened erythrocyte survival will reduce exposure of erythrocytes to glucose with a consequent decrease in HbA1c (%) values, even though the time-averaged blood glucose level may be elevated.[14] Causes of shortened erythrocyte lifetime might be hemolytic anemia or other hemolytic diseases, homozygous sickle cell trait, pregnancy, recent significant or chronic blood loss, etc.
5. In common with other methods, HbA1c values may not accurately reflect mean blood glucose in patients with Hb variants.[15] Glycated HbF (fetal hemoglobin) is not detected as it does not contain the glycated β-chain that characterizes HbA1c. As a consequence, specimens containing high amounts of HbF (>10%) may yield lower than expected HbA1c results. Specimens containing HbS (sickle cell trait) and HbC variants may yield higher than expected HbA1c results. Hb variants may yield viable results depending on the Hb trait, the HbA1c level, and other sample characteristics. In individuals without diabetes or with well controlled diabetes, an average positive bias of 0.5-1.5% is observed with heterozygous HbS (sickle cell trait) and 1.1-2.2% with heterozygous HbC variants. This bias increases with poorly controlled diabetes.[16,17] Other very rare variants in the N-terminal region of the beta chain, HbE, have not been assessed.

Criterion: Recovery within ±10% of initial value.

Icterus	No significant interference.
Lipemia	No significant interference.
Glycemia	No significant interference up to a glucose level of 55.5 mmol/L (1000 mg/dL). A fasting sample is not required.
Other	Acetylated Hb (elevated due to alcohol or aspirin ingestion), carbamylated Hb (elevated in uremia), and labile HbA1c (an intermediate in the formation of stable HbA1c) do not interfere with the test.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Expected values
Protocol 1 (acc. to IFCC):
2.9-4.2% HbA1c[21]
Protocol 2 (acc. to DCCT/NGSP):
4.8-5.9% HbA1c.[21]
HbA1c levels above the established reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer. HbA1c levels may reach 20% or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 8%. Diabetes patients with HbA1c levels below 7% meet the goal of the American Diabetes Association.[18] HbA1c levels below the established reference range may indicate recent episodes of hypoglycemia, the presence of Hb variants, or shortened lifetime of erythrocytes.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data[22]
Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using human samples and controls in an internal protocol (within run n = 20, between run n = 20). The following results were obtained (data based on DCCT/NGSP values):

	Level 1	Level 2
Mean	4.7%	10.3%
CV within run	2.3%	2.2%
CV between run	2.4%	2.4%

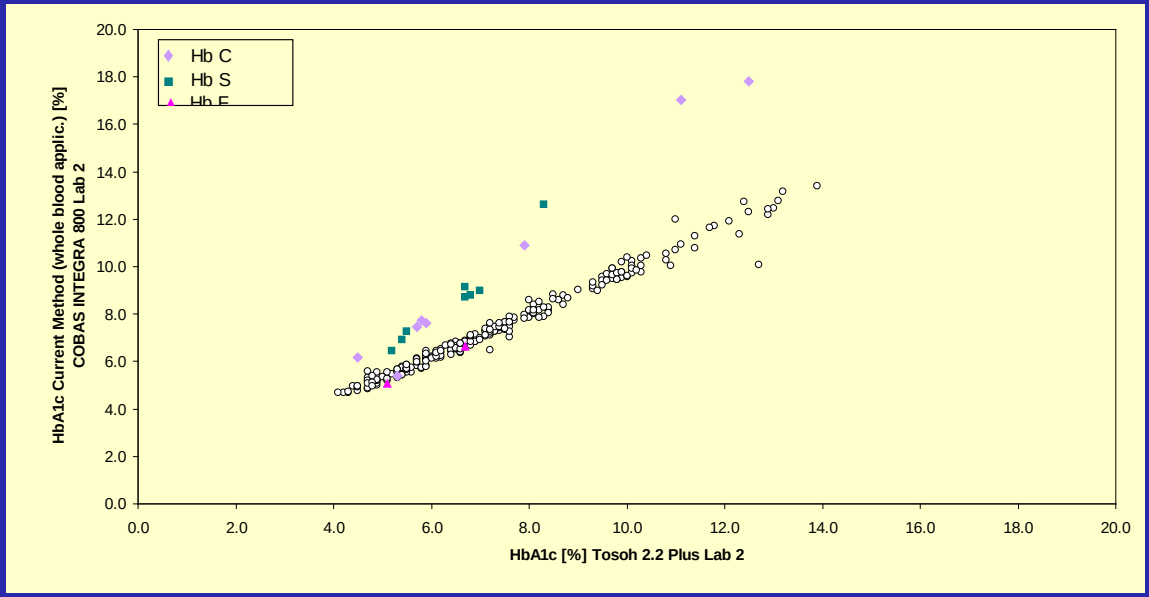
than expected HbA1c results. Specimens containing HbS (sickle cell trait) and HbC variants may yield higher than expected HbA1c results. Hb variants may yield viable results depending on the Hb trait, the HbA1c level, and other sample characteristics. In individuals without diabetes or with well controlled diabetes, an average positive bias of 0.5-1.5% is observed with heterozygous HbS (sickle cell trait) and 1.1-2.2% with heterozygous HbC variants. This bias increases with poorly controlled diabetes.[16,17] Other very rare variants in the N-terminal region of the beta chain, HbE, have not been assessed.

Criterion: Recovery within ±10% of initial value.

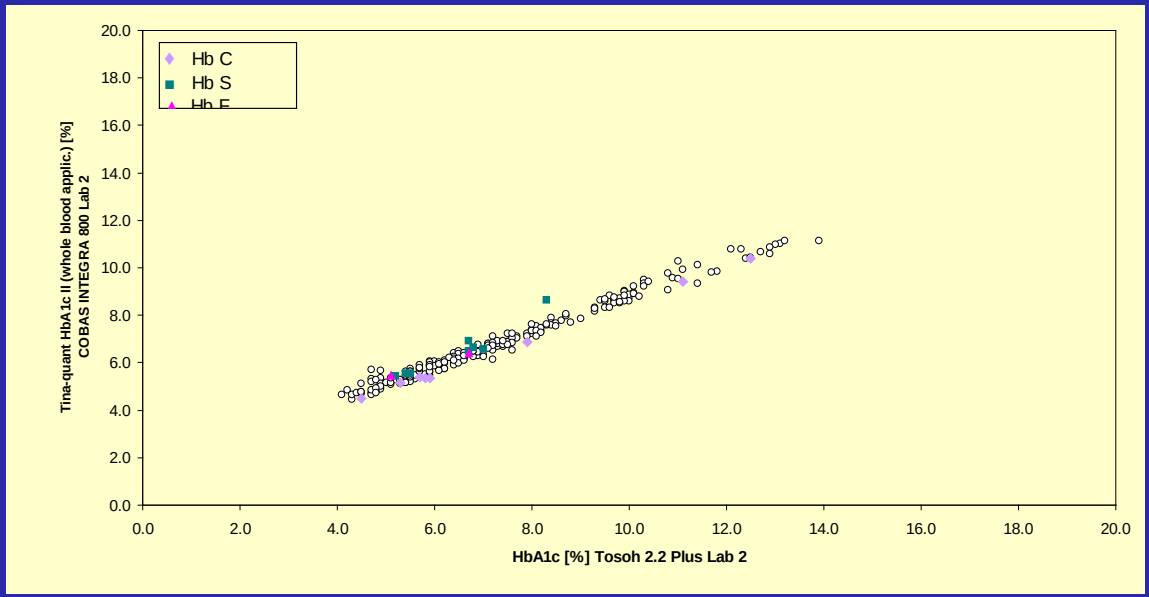
Icterus No significant interference.

www.mylabonline.com, last accessed 6/15/2008

Original Integra Method



"Gen2" Integra Method
Tina-quant A1c



Miedema et al. Clin Chem 2005:51:A234

www.ngsp.org

The following table lists the 20 methods most often used to measure A1C and whether the method is affected by HbC, HbS, HbE or HbD trait or by elevated HbF. Methods are listed in alphabetical order by manufacturer. The criteria used to determine whether or not a method shows interference that is clinically significant (indicated by "Yes") is >±7% at 6 and/or 9% A1C. If your diabetes patient has a hemoglobin variant, your lab should use a method that does not show interference from that variant in order to produce an accurate A1C result.

Method	Interference from HbC	Interference from HbS	Interference from HbE	Interference from HbD	Interference from elevated HbF
Abbott Architect/Aeroset	**Yes**	**Yes**	**@**	**@**	**$**
Arkray ADAMS A1c HA-8180V (Menarini)	No	No	HbA1c not quantified	HbA1c not quantified	No
Axis-Shield Afinion	No	No	No	No	$
Bayer A1cNOW	**Yes**	**Yes**	No	No	$
Beckman AU system	**Yes**	**Yes**	No	No	$
Beckman Synchron System	No	No	No	No	$
Bio-Rad D-10 (A1c program)	No	No	No	No	Yes >10% HbF
Bio-Rad Variant II NU	-	-	No	No	Yes >10% HbF
Bio-Rad Variant II Turbo	No	No	**Yes**	**Yes**	Yes >5% HbF
Bio-Rad Variant II Turbo 2.0	No	No	No/Yes (conflicting reports)	No	Yes >25% HbF

Hemoglobin SC

- Tosoh gave an error message,
  telling us not to trust the apparent A1c,
  because Mr. Donaldson's specimen has no Hemoglobin A
- Integra, at least the original method, has a known limitation,
  yielding falsely elevated values with Hgb S and Hgb C
- Tina-Quant, though, is accurate,
  quantitating all glycated hemoglobins, including S and C
- By Tina-Quant, Mr. Donaldson has a non-diabetic value of 4.8%
- Does he have diabetes at all?

Fingerstick Glucose Results

	Morning Glucose (pre-meal)	Dinnertime Glucose (pre-meal)
Day	mg/dL	mg/dL
1	139	114
2	147	131
3	140	101
4	139	100
5	150	113
6	141	100
7	121	101
8	114	98
9	134	103
10	144	103
11	141	106
12	116	104
13	121	94
14	119	93
15	137	116
16	140	128
17	156	120
18	127	137
19	123	113
20	140	105
21	120	102
22	134	109
23	135	111

➔mild hyperglycemia

based on these data,
estimated mean blood glucose ~120

A1c of 4.8% corresponds to MBG of ~94

eAG of ~91

Analytically Accurate, But Clinically Misleading

- remember, A1c is a time-dependent process
- reference intervals, and indeed MBG calculation, assume normal 120 day RBC lifespan
- RBC lifespan in SC disease is only 29 days!

Blood 1975;45:273-9

- Mr. Donaldson does have diabetes
- A1c cannot be used reliably to assess him
- fingerstick glucose records are needed

Summary: Take Home Points

- Limitations of urine dipstick protein assay:
  - without creatinine, quantitation can be misleading
  - a negative does not rule out BJP
  - a negative does not rule out pathologic microalbuminuria

- For CKD proteinuria screening, including diabetes,
  - make sure correct test is ordered (urine albumin/creatinine)
  - make sure you get the correct answer
    - rule out "hook effect" – dilution, total protein, dipstick protein

- **Issues related to A1c**
  - Sample integrity for glucose: use gray top tubes
  - A1c is a surrogate for frequent fingerstick glucoses
  - beware of analytical limitations as well as RBC lifespan issues

Thank You for Your Attention!