When Accuracy Alone Is Not Sufficient: New Roles for Clinical Chemists

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Southeast Section AACC
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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**
- Choose (and validate) instruments/methods
- Generate accurate, timely results
- Follow doctors’ “orders”
- Plus potentially others

- **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 curable 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
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
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\degree 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 24º 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!!

<table>
<thead>
<tr>
<th>sample</th>
<th>dipstick protein</th>
<th>(estimated) dipstick mg/dL</th>
<th>Chemistry protein mg/dL</th>
<th>creatinine mg/dL</th>
<th>prot/creat ratio</th>
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<td>1+</td>
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<td>2+</td>
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<td>3+</td>
<td>300</td>
<td>358</td>
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<tr>
<td>5</td>
<td>3+</td>
<td>300</td>
<td>358</td>
<td>230</td>
<td>1.6</td>
</tr>
</tbody>
</table>
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
Burtis, CA & Ashwood, ER.
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 spectrophotometry
  - 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

<table>
<thead>
<tr>
<th>method</th>
<th>value from package insert</th>
<th>value in (mg/dL)</th>
<th>sensitivity (relative to dipstick)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipstick</td>
<td>18 mg/dL</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Urine Protein</td>
<td>6 mg/dL</td>
<td>6</td>
<td>3X more sensitive</td>
</tr>
<tr>
<td>Urine Albumin (microalbumin)</td>
<td>3 mg/L</td>
<td>0.3</td>
<td>&gt;50X more sensitive</td>
</tr>
<tr>
<td>Serum Protein</td>
<td>0.2 g/dL</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Serum Albumin</td>
<td>0.1 g/dL</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>method</th>
<th>sensitivity</th>
<th>dilute [creatinine]$_u$ = 20</th>
<th>concentrated [creatinine]$_u$ = 200</th>
</tr>
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<tbody>
<tr>
<td>dipstick</td>
<td>18 mg/dL</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>conventional chemistry</td>
<td>6 mg/dL</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>urine albumin</td>
<td>0.3 mg/dL</td>
<td>15 mg/g</td>
<td>1.5 mg/g</td>
</tr>
</tbody>
</table>
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, 24-hour urine collections were advocated
  - disease threshold was 300 mg/24

- 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?

Hook Effect: A Picture

- free $\lambda$
- intact IgG $\lambda$
- 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**

**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).

<table>
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<tr>
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<th>Assigned value conversion factor</th>
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<tbody>
<tr>
<td>1</td>
<td>200 µL</td>
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<td>0.0</td>
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<tr>
<td>2</td>
<td>1440 µL</td>
<td>20 µL</td>
<td>0.01370</td>
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<tr>
<td>3</td>
<td>860 µL</td>
<td>20 µL</td>
<td>0.02273</td>
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<tr>
<td>4</td>
<td>420 µL</td>
<td>20 µL</td>
<td>0.04545</td>
</tr>
<tr>
<td>5</td>
<td>110 µL</td>
<td>100 µL</td>
<td>0.47619</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>200 µL</td>
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</tr>
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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 with buffer (See below). For further dilution, use 100% solution of NaCl.

**Calculation**
The analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: $mg/dL \times 0.0152 = \mu mol/L$.

**Limitations - Interference**

- **Criteria**: Recovery within ± 10% of initial value.
- **Interference**: 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 500 mg/dL or 188 µmol/L.
- **No interference by acetone**, ascobic 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 urubinogen < 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/dL (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/dL (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/dL (0.046–6.08 µmol/L)

Extended measuring range with rerun: 3–3000 mg/dL (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/dL), 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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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 2600 mg/dL (380 µmol/L).

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Multiply the result obtained by the appropriate dilution factor.
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
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?

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<th>Dec 02</th>
<th>Feb 03</th>
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<th>Oct 03</th>
<th>Jun 04</th>
<th>Jul 05</th>
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<td>154</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>123</td>
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<td>5.4%</td>
<td></td>
<td></td>
<td>4.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1c (Integra)</td>
<td>7.3%</td>
<td>6.8%</td>
<td>6.6%</td>
<td>6.4%</td>
<td>6.6%</td>
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<td></td>
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<tr>
<td>A1c (Tosoh)</td>
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<td>error</td>
<td>error</td>
<td>error</td>
<td>error</td>
<td>error</td>
<td>error</td>
</tr>
</tbody>
</table>

"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) gluoses 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.
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
  - $100 + [(8\%-5\%)\times30] = 100 + (3\times30) = 190$ (my equation)

- versus official equation
  - $MBG = (35.6 \times HbA1c) - 77.3 = 35.6 \times 8 - 77.3 = 207$

Diabetes Care 2002;25:275-278
Translating the A1C Assay Into Estimated Average Glucose Values

David M. Nathan, MD1
Judith Kuenen, MD2
Rikke Borg, MD3
Hui Zheng, PhD1,4

David Schoenfeld, PhD1,4
Robert J. Heine, MD2
for the A1c-Derived Average Glucose (ADAG) Study Group*

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\text{mg/dl} = 28.7 \times \text{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 — A1C levels can be expressed as AG for most patients with type 1 and
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, [A₁c] will decrease by an absolute 2% (or roughly 40%)!

- Rest of the world:

  $$\text{IFCC (mmol/mol)} = (\text{DCCT} (\%) - 2.15) \times 10.929$$

  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
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?

<table>
<thead>
<tr>
<th></th>
<th>Nov 02</th>
<th>Dec 02</th>
<th>Feb 03</th>
<th>Jul 03</th>
<th>Oct 03</th>
<th>Jun 04</th>
<th>Jul 05</th>
</tr>
</thead>
<tbody>
<tr>
<td>fasting glucose</td>
<td>152</td>
<td>154</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>123</td>
</tr>
<tr>
<td>A1c (Hitachi)</td>
<td></td>
<td>5.4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8%</td>
</tr>
<tr>
<td>A1c (Integra)</td>
<td></td>
<td></td>
<td>7.3%</td>
<td>6.8%</td>
<td>6.6%</td>
<td>6.4%</td>
<td>6.6%</td>
</tr>
<tr>
<td>A1c (Tosoh)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>error</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.9%</td>
</tr>
</tbody>
</table>
Chromatogram
(Tosoh 2.2 Plus)

Mr. Donaldson

Typical Patient

BIDMC data
**Not Really “News”**
(I Was Just Unaware of It)

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

<table>
<thead>
<tr>
<th>Method</th>
<th>6% Hb A1c</th>
<th>9% Hb A1c</th>
<th>6% Hb A1c</th>
<th>9% Hb A1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1c 2.2 Plus</td>
<td>−0.10</td>
<td>−0.24</td>
<td>−0.10</td>
<td>−0.54</td>
</tr>
<tr>
<td>DCA 2000</td>
<td>0.26</td>
<td>0.60</td>
<td>0.17</td>
<td>0.52</td>
</tr>
<tr>
<td>Diamat</td>
<td>−0.42</td>
<td>−0.47</td>
<td>0.83b</td>
<td>0.36</td>
</tr>
<tr>
<td>Diastrac</td>
<td>−0.39</td>
<td>−0.94b</td>
<td>−1.28b</td>
<td>−2.06b</td>
</tr>
<tr>
<td>IMX</td>
<td>2.87b</td>
<td>3.17b</td>
<td>0.64</td>
<td>0.36</td>
</tr>
<tr>
<td>Tina-quant II</td>
<td>−0.32</td>
<td>−0.44</td>
<td>−0.19</td>
<td>−0.32</td>
</tr>
<tr>
<td>Variant A1c</td>
<td>0.07</td>
<td>−0.04</td>
<td>0.66b</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*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 the containing Hb C or Hb S trait.

b Both statistically significant (P <0.01) and clinically >0.9% at 6% and 9% Hb A1c, respectively. difference

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

<table>
<thead>
<tr>
<th>Method</th>
<th>Assay principle</th>
<th>6% Hb A1c</th>
<th>9% Hb A1c</th>
<th>6% Hb A1c</th>
<th>9% Hb A1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoBes Integra</td>
<td>Immucassay</td>
<td>2.16b</td>
<td>4.10b</td>
<td>1.45b</td>
<td>2.74b</td>
</tr>
<tr>
<td>Glyco-Tek</td>
<td>Boronate affinity</td>
<td>1.37b</td>
<td>1.45b</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Glycosal</td>
<td>Boronate affinity</td>
<td>0.63b</td>
<td>0.72</td>
<td>0.37</td>
<td>0.55</td>
</tr>
<tr>
<td>HA8140</td>
<td>Ion exchange</td>
<td>0.22</td>
<td>0.28</td>
<td>0.81b</td>
<td>0.57</td>
</tr>
<tr>
<td>Nyocard</td>
<td>Boronate affinity</td>
<td>0.23</td>
<td>0.07</td>
<td>−0.13</td>
<td>−0.13</td>
</tr>
<tr>
<td>Synchro CX7</td>
<td>Immucassay</td>
<td>−0.52</td>
<td>−0.27</td>
<td>−0.41</td>
<td>−0.19</td>
</tr>
<tr>
<td>Variant II</td>
<td>Ion exchange</td>
<td>0.42</td>
<td>0.65</td>
<td>0.57</td>
<td>0.43</td>
</tr>
<tr>
<td>Variant GHB</td>
<td>Boronate affinity</td>
<td>0.59</td>
<td>0.86</td>
<td>0.40</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* 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 A1c at 6% and 9% Hb A1c, respectively) differences were found.

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

Roche Acknowledges the Issue in Integra Package Insert
Original Integra Method

“Gen2” Integra Method
Tina-quant A1c

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.

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

<table>
<thead>
<tr>
<th>Day</th>
<th>Morning Glucose (pre-meal) mg/dL</th>
<th>Dinnertime Glucose (pre-meal) mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139</td>
<td>114</td>
</tr>
<tr>
<td>2</td>
<td>147</td>
<td>131</td>
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<tr>
<td>3</td>
<td>140</td>
<td>101</td>
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<td>4</td>
<td>139</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>113</td>
</tr>
<tr>
<td>6</td>
<td>141</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>121</td>
<td>101</td>
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<td>22</td>
<td>134</td>
<td>109</td>
</tr>
<tr>
<td>23</td>
<td>135</td>
<td>111</td>
</tr>
</tbody>
</table>

→ 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!
- 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 gluoses
  - beware of analytical limitations as well as RBC lifespan issues
Thank You for Your Attention!