Laboratory Interpretive Services

TAking your lab to the next level in meeting medical needs

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Professor of Pathology, Microbiology and Immunology
Medical Director, Clinical Chemistry and Point-of-Care Testing

4/22/2020
Learning Objectives

I. Recognize the complexity of physician ordering to select the right test for the patient

II. Identify how to provide an interpretive service

III. Describe the benefits of a Laboratory Formulary Committee
Has the right test been ordered?

Ordering

Collection

Identification

Transportation

Preparation

Error between result receipt and action?

Action

Interpretation

Reporting

Analysis

The nine steps in the performance of any laboratory test. The brain-to-brain TAT loop.

Lundberg, 1981 JAMA
Educational Mismatch with Medical Practice Competency

What medical students are taught about the diagnostic tests they will use in practice:

- Anatomic pathology tests
- Radiology tests
- Clinical laboratory tests

What diagnostic tests doctors order in practice and are required to interpret the test results by themselves:

- Anatomic pathology tests
- Radiology tests
- Clinical laboratory tests

NUMBER OF HOURS MEDICAL STUDENTS SPEND LEARNING AP: 60 – 300
NUMBER OF HOURS MEDICAL STUDENTS SPEND LEARNING LABORATORY MEDICINE: 9

*Brian Smith and CLIHC group CDC preliminary data from survey US Medical Schools*
The Clinician’s Diagnostic Challenge

- Radiologists do not give MRI images back to ordering physician without interpretation
- Anatomic pathologists do not give biopsies back to surgeons without interpretation

Why is it acceptable for clinical labs to give complex lab results back to physicians without interpretation?
Ordering Confusion

- Vitamin D
- Vitamin D2
- Vitamin D3

- 25 hydroxy vitamin D
- 25-OH vitamin D
- 25 hydroxy vitamin D2
- 25-OH vitamin D2
- 25 hydroxy vitamin D3
- 25-OH vitamin D3

- 1,25 dihydroxy vitamin D
- 1,25-diOH vitamin D
- 1,25 dihydroxy vitamin D2
- 1,25-diOH vitamin D2
- 1,25 dihydroxy vitamin D3
- 1,25-diOH vitamin D3

Multiple abbreviations and variations on naming

A doctor wants to know if a patient has vitamin D deficiency – which single test do they order?
Consequences of Clinical Confusion

DOCTORS MISS THE NECESSARY TESTS

Laboratory testing is an integral part of clinical decision-making

• (29 – 98% of medical encounters order 1 or more laboratory tests, depending on the medical specialty – Outpatient 29%, ED 56%, inpatient 98%) JALM 2017;01:410-14.

Doctors order the wrong tests


Many approaches emerge to diagnose same condition

• 30% of care delivered in the US is inappropriate. ASCP Choosing wisely campaign

AREAS WHERE INTERPRETIVE ASSISTANCE NEEDED

- Hepatitis serologies
- Coagulation
- Drug testing
- Infectious disease
Diagnostic Management Team (DMT)

• Physicians order tests by requesting DMT evaluation
  o Abnormal screening test
  o Clinical sign or symptom

• Expert team in the DMT synthesizes the clinical and laboratory data to provide a concise interpretive narrative based upon medical evidence
# 4 Pillars of a Diagnostic Management Team

<table>
<thead>
<tr>
<th>Complex testing</th>
<th>Context-driven test selection</th>
<th>Transparency</th>
<th>Interpretive reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>• DMT clarifies unfamiliar test choices – through initial guidance or reflexive orders</td>
<td>• Tests are chosen by group of laboratorians/clini cians in real-time based on clinical context (assists in best practice/guideline driven test selection)</td>
<td>• DMT complements clinician’s understanding of their patient’s disease process</td>
<td>• Interpretation ties the lab findings to clinical context to bring the findings together</td>
</tr>
</tbody>
</table>
Diagnosis and Treatment of Anemia

- Complex differential for anemia
  - Bleeding
  - Cancer/Leukemia
  - Age
  - Chronic disease
  - Iron deficiency
  - Hemoglobin variant

- Hematologists observe cell differential, but don’t consider chemistry tests – chemists don’t review heme results.
  - Requires clinician to synthesize multiple results to develop a diagnosis

- A Heme DMT can review chemistry, hematology and other laboratory results to synthesize one combined interpretation that assist clinician in differential diagnosis of anemia and ongoing patient care
Heme DMT

Clinical components
• Medical and family history
• Medications (hydroxyurea)
• Transfusion history

Analytical components
• HPLC (with reflex electrophoresis for hemoglobin variant)
• CBC (Hgb, RBC parameters, Mentzer’s index, SickleDex)
• Iron studies (Iron, TIBC, % saturation, ferritin)
• Newborn screen results
Mentzer’s Index

- If CBC shows microcytic anemia, ratio of MCV/RBC can distinguish iron deficiency from thalassemia.
- < 13 beta thalassemia more likely (thalassemia is a disorder of globin synthesis, normal amount of cells, but cells produced are smaller and more fragile, RBC normal, but MCV down, so ratio is low)
- >13 iron deficiency more likely (in iron deficiency bone marrow can’t produce as many cells and cells are small, both MCV and RBC down)
- Not 100% reliable, iron deficiency and beta thalassemia can coexist, and ferritin more reliable measure of iron deficiency
### BioRad Variant II

**β-Thal Short Program**

<table>
<thead>
<tr>
<th>Peak name</th>
<th>Retention time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>F window</td>
<td>0.98–1.20</td>
</tr>
<tr>
<td>A&lt;sub&gt;0&lt;/sub&gt; window</td>
<td>1.90–3.10</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt; window</td>
<td>3.30–3.90</td>
</tr>
<tr>
<td>D window</td>
<td>3.90–4.30</td>
</tr>
<tr>
<td>S window</td>
<td>4.30–4.70</td>
</tr>
<tr>
<td>C window</td>
<td>4.90–5.30</td>
</tr>
</tbody>
</table>

![Chromatogram](image.png)

- **Standard**
- **Hb A1c**: 1.16
- **Hb E**: 4.45
- **Hb D**: 4.45
<table>
<thead>
<tr>
<th>Variant name</th>
<th>n⁶</th>
<th>Retention time,⁵ min</th>
<th>%HB⁺</th>
<th>ΔTime,⁴ months</th>
<th>Variant⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Barts</td>
<td>ND⁷</td>
<td>0.2</td>
<td>ND</td>
<td>32</td>
<td>γ⁴</td>
</tr>
<tr>
<td>Hb H</td>
<td>12</td>
<td>0.2</td>
<td>12.5 (4.0)</td>
<td>32</td>
<td>β⁴</td>
</tr>
<tr>
<td>Hb F</td>
<td>ND</td>
<td>0.5</td>
<td>ND</td>
<td>32</td>
<td>–</td>
</tr>
<tr>
<td>Hb F</td>
<td>160⁺</td>
<td>1.60 (0.017)</td>
<td>1.0 (0.5)</td>
<td>32</td>
<td>β 135Gly→Asp</td>
</tr>
<tr>
<td>Hb Cardinal</td>
<td>2</td>
<td>1.50:1.48</td>
<td>52.4:49.3</td>
<td>5</td>
<td>β 131Gln→Glu</td>
</tr>
<tr>
<td>Hb J/oxford</td>
<td>1</td>
<td>1.60</td>
<td>24.7</td>
<td>–</td>
<td>α 15Gly→Asp</td>
</tr>
<tr>
<td>Hb Austin</td>
<td>3</td>
<td>1.68 (0.017)</td>
<td>47.1 (0.4)</td>
<td>12</td>
<td>β 40Arg→Ser</td>
</tr>
<tr>
<td>Hb NBaltimore</td>
<td>6</td>
<td>1.70 (0.031)</td>
<td>47.8 (0.9)</td>
<td>21</td>
<td>β 95Lys→Glu</td>
</tr>
<tr>
<td>Hb Fukuyama</td>
<td>2</td>
<td>1.72:1.73</td>
<td>−</td>
<td>0</td>
<td>β 77His→Tyr</td>
</tr>
<tr>
<td>Hb Fannin/Lubbock</td>
<td>7</td>
<td>1.75 (0.024)</td>
<td>35.0 (3.0)</td>
<td>21</td>
<td>β 119Gly→Asp</td>
</tr>
<tr>
<td>Hb J/antiole</td>
<td>2</td>
<td>1.75:1.75</td>
<td>19.6:21.2</td>
<td>0.5</td>
<td>α 61Lys→Thr</td>
</tr>
<tr>
<td>Hb J/Mexico</td>
<td>2</td>
<td>1.74:1.78</td>
<td>22.7:22.3</td>
<td>10</td>
<td>α 54Gln→Glu</td>
</tr>
<tr>
<td>Hb J/Meurant</td>
<td>2</td>
<td>1.88:1.88</td>
<td>26.4:26.2</td>
<td>11</td>
<td>α 120Ala→Glu</td>
</tr>
<tr>
<td>Hb J/roan</td>
<td>1</td>
<td>1.84</td>
<td>−</td>
<td>–</td>
<td>α 5A→Asp</td>
</tr>
<tr>
<td>Hb J/Bangkok</td>
<td>1</td>
<td>2.02</td>
<td>43.6</td>
<td>–</td>
<td>β 56Gly→Asp</td>
</tr>
<tr>
<td>Hb J/Ty-Gard</td>
<td>1</td>
<td>2.20</td>
<td>34.1</td>
<td>–</td>
<td>β 124Pro→Gln</td>
</tr>
<tr>
<td>Hb Kfl¹¹</td>
<td>2</td>
<td>2.26:2.26 (4.93; 4.87)</td>
<td>26.8:23.5 (7.0; 7.3)</td>
<td>24</td>
<td>β 90Val→Met</td>
</tr>
<tr>
<td>Hb A²</td>
<td>160⁺</td>
<td>2.43 (0.041)</td>
<td>86.3 (1.5)</td>
<td>32</td>
<td>–</td>
</tr>
<tr>
<td>Hb New York</td>
<td>Δ⁶</td>
<td>2.43 (0.010)</td>
<td>Does not separate</td>
<td>12</td>
<td>β 113Val→Glu</td>
</tr>
<tr>
<td>Hb Twin Peaks</td>
<td>3</td>
<td>Appears as hump</td>
<td>Does not separate</td>
<td>11</td>
<td>α 113Leu→His</td>
</tr>
<tr>
<td>Hb Lepore</td>
<td>3</td>
<td>3.37 (0.019)</td>
<td>12.1 (1.5)</td>
<td>24</td>
<td>δ/β hybrid</td>
</tr>
<tr>
<td>Hb D/Fran</td>
<td>1</td>
<td>3.49</td>
<td>47.7</td>
<td>–</td>
<td>β 22Glu→Gln</td>
</tr>
<tr>
<td>Hb A²</td>
<td>160⁺</td>
<td>3.63 (0.035)</td>
<td>2.7 (0.4)</td>
<td>32</td>
<td>β 26Glu→Lys</td>
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<tr>
<td>Hb E</td>
<td>83⁺</td>
<td>3.69 (0.069)</td>
<td>30.3 (4.0)</td>
<td>32</td>
<td>β 52Asp→Asn</td>
</tr>
<tr>
<td>Hb Osu Christiansborg</td>
<td>1</td>
<td>3.77</td>
<td>44.0</td>
<td>–</td>
<td>α 30Glu→Gln</td>
</tr>
<tr>
<td>Hb G/Honolulu</td>
<td>1</td>
<td>3.86</td>
<td>27.4</td>
<td>–</td>
<td>α 30Glu→Gln</td>
</tr>
<tr>
<td>Hb Korle-Bu</td>
<td>8</td>
<td>3.92 (0.050)</td>
<td>46.5 (3.7)</td>
<td>16</td>
<td>β 73Asp→Asn</td>
</tr>
<tr>
<td>Hb DPunab</td>
<td>7</td>
<td>4.18 (0.007)</td>
<td>33.1 (1.8)</td>
<td>24</td>
<td>β 121Glu→Glu</td>
</tr>
<tr>
<td>Hb G/Philadelphia</td>
<td>8</td>
<td>4.22 (0.037)</td>
<td>26.4 (6.6)</td>
<td>29</td>
<td>α 66Asn→Lys</td>
</tr>
<tr>
<td>Hb E/Saskatoon</td>
<td>2</td>
<td>4.34:4.32</td>
<td>39.3:40.4</td>
<td>2</td>
<td>β 22Glu→Lys</td>
</tr>
<tr>
<td>Hb S</td>
<td>3587⁺⁺⁺</td>
<td>4.51 (0.030)</td>
<td>34.9 (4.1)</td>
<td>32</td>
<td>β 60Lys→Val</td>
</tr>
<tr>
<td>Hb Manitoba</td>
<td>1</td>
<td>4.58</td>
<td>16.5</td>
<td>–</td>
<td>α 102Ser→Arg</td>
</tr>
<tr>
<td>Hb Montgomery</td>
<td>7</td>
<td>4.58 (0.020)</td>
<td>15.7 (2.2)</td>
<td>32</td>
<td>α 48Lys→Arg</td>
</tr>
<tr>
<td>Hb A²</td>
<td>81⁺</td>
<td>4.59 (0.030)</td>
<td>1.2 (0.1)</td>
<td>1</td>
<td>δ 16Gly→Arg</td>
</tr>
<tr>
<td>Hb G/Thailand</td>
<td>1</td>
<td>4.67</td>
<td>29.3</td>
<td>–</td>
<td>α 74Asp→His</td>
</tr>
<tr>
<td>Hb Hassaroon</td>
<td>7</td>
<td>4.83 (0.016)</td>
<td>17.8 (1.5)</td>
<td>32</td>
<td>α 47Asp→His</td>
</tr>
<tr>
<td>Hb G/Arab</td>
<td>6</td>
<td>4.91 (0.008)</td>
<td>35.5 (3.0)</td>
<td>24</td>
<td>β 121Glu→Lys</td>
</tr>
<tr>
<td>Hb G/Sumatra</td>
<td>1</td>
<td>5.08</td>
<td>24.2</td>
<td>–</td>
<td>β 70Lys→Lys</td>
</tr>
<tr>
<td>Hb C</td>
<td>965⁺⁺⁺</td>
<td>5.16 (0.013)</td>
<td>35.6 (4.0)</td>
<td>32</td>
<td>β 60Lys→Lys</td>
</tr>
</tbody>
</table>

* Number of observations.
* Mean (SD) relative retention time except when n was <3, for which the individual retention times are given.
* Mean (SD) percentage of the hemoglobin variant as a fraction of the total hemoglobin.

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Isoelectric Focusing (IEF)
Case

- 33 mo male, unknown ethnicity with anemia (9.7 Hgb), microcytosis (small cells), anisocytosis (unequal size). Mentzer’s index = 13.4 (indeterminate - > 13 Fe deficiency < 13 Thalassemia)

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>9.6%*</td>
<td></td>
<td>1.07</td>
<td>171503</td>
</tr>
<tr>
<td>F2</td>
<td>3.6</td>
<td></td>
<td>1.30</td>
<td>546255</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.6</td>
<td></td>
<td>1.46</td>
<td>203651</td>
</tr>
<tr>
<td>F1</td>
<td>4.3</td>
<td></td>
<td>1.69</td>
<td>271132</td>
</tr>
<tr>
<td>A0</td>
<td>77.3</td>
<td></td>
<td>2.33</td>
<td>1306411</td>
</tr>
<tr>
<td>A2</td>
<td>4.9%*</td>
<td></td>
<td>3.62</td>
<td>52159</td>
</tr>
</tbody>
</table>

Total Area: 1,793,609

F Concentration = 9.6%*
A2 Concentration = 4.9%*

*Values outside of expected ranges

Analysis comments: HbF = 9.6%* (<2%), HbA = 85.5%* (>90%), HbA2 = 4.9%* (2.5 – 3.9%)
- **Microcytosis** – small red blood cells
- **Anisocytosis** – RBCs of varying size

**HEMOGLOBIN VARIANT EVALUATION**

<table>
<thead>
<tr>
<th>HEMOGLOBIN</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>85.5 %</td>
</tr>
<tr>
<td>A2</td>
<td>4.9 %</td>
</tr>
<tr>
<td>F</td>
<td>9.6 %</td>
</tr>
</tbody>
</table>

**Note:** The hemoglobin profile shows an elevated hemoglobin F and A2. Given the elevated hemoglobin A2 and the patient’s peripheral smear findings (microcytosis and mild anisocytosis), these findings are suggestive of beta-thalassemia trait in the appropriate clinical context.

Electronically signed out by:
Aaron C. Shaver, N.D., Ph.D.

The sample was analyzed by High Performance Liquid Chromatography (HPLC) and the relative mobility of the patient’s sample was compared with that of hemoglobins A, F, S, C, D and E.

**HGB EVAL INTERPRETATION**
An Isoelectric Focusing (IEF) plate was performed and evaluated.
Reasons for Requesting Hemoglobin Variant Analysis

Anemia of unknown origin in ethnic patient

Follow-up to abnormal newborn screen

Adoption

Athletic exam for competitive sports

Prenatal screening – patients of ethnic origin
Endocrine HbA1c DMT

HPLC ANALYSIS THAT FAILS A DELTA CHECK OR >15% FLAG

Clinical/Analytical components:
- Medical history
- Diagnosis of diabetes
- Prior HbA1c result
- Drugs/medications
- Recent transfusions
- Hemoglobin variant
Appendix 1: Flow Diagram for A1C Chromatogram Evaluation

Total Area Between 1,000,000 and 3,500,000

- Yes
  - Labile A1c > 6%
    - Yes
      - Repeat after 24 hours at room temperature
    - No
      - Carbamylated A1c > 4%
        - No
          - Re-dilute and repeat
  - No
    - Comment “A1CFH”. No result.

- Hb F > 25%
  - Yes
    - Hold result and leave copy of chromatogram for fellow to review.
  - No
    - HbA1c < 3.5%
      - Yes
        - HbA1c < 3.5%
          - No
            - HbA1c ≥ 15%
              - Yes
                - Hold result and leave copy of chromatogram for fellow to review.
              - No
                - Hb variant present? (check variant & C-windows)
                  - Yes
                    - Variant area > 40% or < 20%
                      - Yes
                        - Run on β-thal program, leave chromatograms for fellow to review.
                      - No
                        - HgA > 40%
                          - Yes
                            - P3 or P4 > 10%
                              - Yes
                                - Cancel, recollect specimen
                              - No
                                - P3 > 5% or P4 > 10%
                                  - Yes
                                    - Report A1c
                                  - No
                                    - Cancel, recollect specimen
                          - No
                            - P3 or P4 > 10%
                              - Yes
                                - Cancel, recollect specimen
                              - No
                                - Report A1c
                  - No
                    - P3 or P4 > 10%
                      - Yes
                        - Cancel, recollect specimen
                      - No
                        - Report A1c

Revised May 2018

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Inappropriately Low HbA1c  |  Inappropriately High HbA1c  |  Variable Effect on HbA1c+
---|---|---
• Hemolysis  |  • Iron deficiency  |  • Fetal hemoglobin
• Certain hemoglobinopathies  |  • Vitamin B12 deficiency  |  • Methemoglobin
• Recent blood transfusion  |  • Alcoholism  |  • Certain hemoglobinopathies
• Acute blood loss  |  • Uremia  |  • Hyperbilirubinemia
• Hypertriglyceridemia  |  • Drugs*  |  
• Drugs*  |  
• Chronic liver disease  |  

*Refer text and Table 2 + method-dependent

---

<table>
<thead>
<tr>
<th>Postulated Mechanism</th>
<th>Falsely Low HbA1c</th>
<th>Falsely High HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antiretrovirals[18]</td>
<td>Trimethoprim-</td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole[14]</td>
<td></td>
</tr>
<tr>
<td>Altered hemoglobin</td>
<td>Hydroxyurea[19]</td>
<td></td>
</tr>
<tr>
<td>Altered glycation</td>
<td>Vitamin C[19]</td>
<td>Vitamin E[19]</td>
</tr>
<tr>
<td></td>
<td>Aspirin (small doses)[20]</td>
<td></td>
</tr>
<tr>
<td>Interference with assays</td>
<td></td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(large doses)[20]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chronic opiate use[21]</td>
</tr>
<tr>
<td>Peak Name</td>
<td>NGSP %</td>
<td>Area %</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>A1a</td>
<td>---</td>
<td>1.0</td>
</tr>
<tr>
<td>A1b</td>
<td>---</td>
<td>0.6</td>
</tr>
<tr>
<td>F</td>
<td>---</td>
<td>0.7</td>
</tr>
<tr>
<td>LA1c</td>
<td>---</td>
<td>1.4</td>
</tr>
<tr>
<td>A1c</td>
<td>4.2</td>
<td>---</td>
</tr>
<tr>
<td>P3</td>
<td>---</td>
<td>4.6</td>
</tr>
<tr>
<td>P4</td>
<td>---</td>
<td>0.7</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>67.5</td>
</tr>
</tbody>
</table>

Total Area: 1,670,521

HbA1c (NGSP) = 4.2 %

DOB: 19-146-0024138
Report Generated: 06/29/2019 13:15:02
Operator ID: NR

- Recent transfusion
  - HbA1c "not accurate due to recent transfusion"
- DO NOT report
- Dapsone
  - Unable to report HbA1c result due to interference from the medication Dapsone

N 5/28/2019

DOB: "G" male white, age 54. Diagnosed with Type 1 diabetes, well controlled, diabetes prior 9/2/19 = 8.4 on DAPSONE 150/150 and transfused on 5/9.
Prenatal Laboratory Testing

- American College of Obstetricians and Gynecologists (ACOG) recommend hemoglobin variant screening on high-risk populations, including individuals of African, Southeast Asian and Mediterranean ancestry.

- Testing is not recommended in low-risk ethnic groups such as Caucasians, Japanese, Native Americans, Inuit and Koreans.

<table>
<thead>
<tr>
<th>Test</th>
<th>ALL PREGNANT WOMEN</th>
<th>SPECIFIC HIGH-RISK POPULATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood type and screen</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CBC with platelet count</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Urine culture and sensitivity</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B surface antigen</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Rubella screening</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>HIV testing</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Syphilis screening</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Chlamydia screening</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis carrier screening</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Gonorrhea screening</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Gestational diabetes screening</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Hemoglobinopathy screening</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Prenatal Hemoglobin Variant Orders

• We noted an unusual number of hemoglobin variant test orders on prenatal patients from low-risk ethnicities – contrary to ACOG guidelines, primarily from 4 OB/GYN clinics

• In these clinics, orders were directed by electronic order set – automatically orders hemoglobin variant screening on all patients, regardless of ethnicity, at their first prenatal visit

• This workflow requires physician to remove the hemoglobin variant test from low risk patients – an added step that is often missed

• We updated physician order sets to better align to ACOG guidelines – removed hemoglobin variant test as part of order set

• Physician education for changed workflow to remember to add the hemoglobin variant test for high risk ethnic groups
Impact of Improved Utilization

Before order set change

Caucasian 31% Other 69%
App. 36 orders per month

Pre = July 2014 – Jan 2015
Post = Jan 2015 – May 2015

After order set change

Caucasian 4% (App. 1 order per month)
Other 96%

Orders placed prior to order set change
- Patient mentioned possible family history of thalassemia
- Order placed erroneously by resident physician
- 2 Patients
- 1 patient
- 1 patient

Figure 2: Decrease in the number of hemoglobin variant screening orders in pregnant Caucasian women following removal from the order set

<table>
<thead>
<tr>
<th>OB/GYN HEMOGLOBIN VARIANT SCREENING TEST VOLUME/MONTH</th>
<th>ASSOCIATED COST *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before order set change</td>
<td>36</td>
</tr>
<tr>
<td>Patient cost/test $120</td>
<td></td>
</tr>
<tr>
<td>($120 X 36 = $4,320)</td>
<td></td>
</tr>
<tr>
<td>Reagent cost/test $12.15</td>
<td></td>
</tr>
<tr>
<td>($12.15 X 36 = $437.40)</td>
<td></td>
</tr>
<tr>
<td><strong>Total monthly cost = $4,757.40</strong></td>
<td></td>
</tr>
<tr>
<td>After order set change</td>
<td>1</td>
</tr>
<tr>
<td>Patient cost/test $120</td>
<td></td>
</tr>
<tr>
<td>($120 X 1 = $120)</td>
<td></td>
</tr>
<tr>
<td>Reagent cost/test $12.15</td>
<td></td>
</tr>
<tr>
<td>($12.15 X 1 = $12.15)</td>
<td></td>
</tr>
<tr>
<td><strong>Total monthly cost = $132.15</strong></td>
<td></td>
</tr>
</tbody>
</table>

* Additional costs associated with this test include labor costs for technologist to run and report the test, as well as resident/fellow and medical director time to review results

Estimated annual savings of $55,503
Reasons for Ordering Toxicology DMT

- Pain management compliance
- Screening for drugs-of-abuse
- Outpatient ADHD medication compliance
- Cancer and transplantation use of cannabis
- Interpretation of DAU test results
Toxicology DMT

Clinical components

• Clinical question being asked!
• Medical history
• Medication history

Analytical components

• Immunoassay screen results (raw data)
• Mass spectrometry results (quantitative)
• Urine creatinine/osmolality or signs of adulteration
• Urinalysis if available (specific gravity)
After Oxycodeone use, urine may be positive for:
» Oxycodeone only
» Oxycodeone and Oxymorphone
» Oxymorphone only

Hydrocodone can be present at very high Oxycodeone concentrations because Hydrocodone can be an impurity in the manufacturing process.

After Oxymorphone use, urine should be positive for Oxymorphone.

Hydrocodone is a trace metabolite of Codeine. Although rarely detected, it may be present if Codeine concentrations are very high.

After Morphine use, urine may be positive for Morphine. Hydromorphone is a trace metabolite of Morphine found only when very high levels of Morphine are present.
Drugs Not Prescribed in Patient’s Sample

- The laboratory was asked to provide an interpretation of the presence of hydrocodone and dihydrocodeine in this patient's specimen.
- The patient is prescribed oxycodone.
- Semi-quantitative results by mass spectrometry were 3030 ng/mL for oxycodone, 854 ng/mL for oxymorphone, 213 ng/mL for hydrocodone, and 23 ng/mL for dihydrocodeine.
- Hydrocodone can be seen in small amounts in prescription oxycodone.
- Dihydrocodeine is a metabolite of hydrocodone.
- The relative concentrations of the analytes are consistent with use of oxycodone.
Case

• Physician calls regarding interpretation of DAU test results

• Patient is on OxyIR (oxycodone intermediate release), #180/mo.

• Drug test screen results:
  • Opiate screen positive (>30,000 ng/mL reactivity),
  • Oxycodone screen negative (<300 ng/mL)

• Drug MS confirmation results:
  • Morphine (50,000 ng/mL)
  • Hydromorphone (120 ng/mL).
Heroin
(Metabolized too quickly to be detected)

Small amounts of codeine in urine samples can result from codeine impurities in heroin manufacturing; NOT due to metabolism.

6-MAM
(6-monoacetylmorphine)
- Metabolite unique to Heroin
- Remains in urine <24 hours

Morphine
(Avinza®, Kadian®, MS Contin®, MSIR®, Roxanol®, Embedda®)

Minor metabolite of Morphine.

Hydrocodone
(Lorcet®, Lortab®, Norco®, Vicodin®, Vicoprofen®)

Hydromorphone
(Dilaudid®)

Oxycodone
(OxyContin®, Percocet, OxyIR®, Percodan®, Roxicodone®, Tylox®)

Oxymorphone
(Opana ER®, Opana IR®, Numorphan®)
Laboratory Interpretation

- On review of results, screening immunoassay was positive (>30,000 ng/mL reactivity) to opiate class and negative for oxycodone reactivity.

- Confirmation by GC/MS demonstrated the presence of morphine (50,000 ng/mL) and hydromorphone (120 ng/mL).

- These results are more consistent with the use of morphine than oxycodone, as trace amounts of hydromorphine can be seen with higher concentrations of morphine.

- Please contact lab if further questions.
Amino Acid Metabolism

• Humans are unable to synthesize all twenty AA needed for protein synthesis.
• AA which cannot be synthesized must then be acquired via the diet (essential AA).
• Dietary intake of AA is typically not balanced to exactly match the body's demands. Dietary AA must be chemically modified and rearranged to provide adequate levels of all the AA needed.
• Large number of pathways in the body for balancing the pool of AA, both for synthesis and for degradation. The number of enzymes involved creates a great potential for genetic diseases. Disruption (by mutation of just one enzyme) in the metabolism of only one AA can have profound consequences for growth and development; some of the genetic diseases are fatal.
Amino Acids

**Essential amino acids**
- Essential amino acids cannot be made by the body. As a result, they must come from food.
- The nine essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

**Nonessential amino acids**
- "Nonessential" means that our bodies produce an amino acid, even if we don't get it from the food, we eat.
- They include: alanine, asparagine, aspartic acid, and glutamic acid.

**Conditional amino acids**
- Conditional amino acids are usually not essential, except in times of illness and stress.
- They include: arginine, cysteine, glutamine, tyrosine, glycine, ornithine, proline, and serine.
# Reasons for Requesting Inborn Errors of Metabolism DMT

<table>
<thead>
<tr>
<th>Newborn</th>
<th>Child/adolescent</th>
<th>Nutritional support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure to thrive</td>
<td>Developmental delay</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>Vomiting, seizure disorders</td>
<td></td>
</tr>
<tr>
<td>Abnormal newborn screen</td>
<td>Autism spectrum and behavioral disorders</td>
<td></td>
</tr>
</tbody>
</table>
Inborn Errors of Metabolism DMT

Clinical components
• Medical and family history
• Medications
• Nutrition and diet

Analytical components
• Newborn screen
• Past AA, organic acids, acylcarnitine or genetic test results
• Plasma, Urine or CSF amino acid analysis
Cystine = oxidized dimer of cysteine
Interpretation: Bailey is a 6 year old female with homocystinuria, on treatment. The plasma amino acid profile shows a minor elevation in methionine. Free homocystine was not detected on this profile.

Plasma Amino Acids:
Alanine: 448 mc mol/L (Ref: 152-547)  
Aminobutyric Acid: 8 mc mol/L (Ref: 4-31)  
Arginine: 66 mc mol/L (Ref: 10-140)  
Asparagine: 50 mc mol/L (Ref: 23-112)  
Aspartic Acid: 5 mc mol/L (Ref: 1-24)  
Citrulline: 34 mc mol/L (Ref: 1-48)  
Cystine: 19 mc mol/L (Ref: 5-45)  
Glutamic Acid: 15 mc mol/L (Ref: 10-133)  
Glutamine: 730 mc mol/L (Ref: 254-823)  
Glycine: 285 mc mol/L (Ref: 127-341)  
Histidine: 73 mc mol/L (Ref: 41-125)  
Hydroxyproline: 21 mc mol/L (Ref: 3-45)  
Iso-Leucine: 49 mc mol/L (Ref: 22-107)  
Leucine: 80 mc mol/L (Ref: 49-216)  
Lysine: 67 mc mol/L (Ref: 48-284)  
Methionine: 51 mc mol/L (Ref: 7-47)  
Ornithine: 54 mc mol/L (Ref: 10-162)  
Phenylalanine: 49 mc mol/L (Ref: 26-91)  
Proline: 204 mc mol/L (Ref: 59-369)  
Serine: 132 mc mol/L (Ref: 69-187)  
Taurine: 55 mc mol/L (Ref: 10-170)  
Threonine: 71 mc mol/L (Ref: 35-226)  
Tryptophan: 47 mc mol/L (Ref: 31-79)  
Tyrosine: 56 mc mol/L (Ref: 24-115)  
Valine: 149 mc mol/L (Ref: 74-321)  

Other Amino Acid: free homocystine = 0 mc mol/L

Interpretation: Brandon is an 11 year old male with homocystinuria, on treatment. The plasma amino acid profile shows an elevated methionine. Free homocystine was not detected on this profile.

Plasma Amino Acids:
Alanine: 208 mc mol/L (Ref: 152-547)  
Aminobutyric Acid: 13 mc mol/L (Ref: 4-31)  
Arginine: 74 mc mol/L (Ref: 10-140)  
Asparagine: 37 mc mol/L (Ref: 23-112)  
Aspartic Acid: 6 mc mol/L (Ref: 1-24)  
Citriulline: 36 mc mol/L (Ref: 1-46)  
Cystine: 28 mc mol/L (Ref: 5-45)  
Glutamic Acid: 28 mc mol/L (Ref: 10-133)  
Glutamine: 591 mc mol/L (Ref: 254-823)  
Glycine: 195 mc mol/L (Ref: 127-341)  
Histidine: 74 mc mol/L (Ref: 41-125)  
Hydroxyproline: 22 mc mol/L (Ref: 3-45)  
Iso-Leucine: 58 mc mol/L (Ref: 22-107)  
Leucine: 184 mc mol/L (Ref: 49-216)  
Lysine: 129 mc mol/L (Ref: 48-284)  
Methionine: 74 mc mol/L (Ref: 7-47)  
Ornithine: 51 mc mol/L (Ref: 10-162)  
Phenylalanine: 48 mc mol/L (Ref: 26-91)  
Proline: 127 mc mol/L (Ref: 59-369)  
Serine: 79 mc mol/L (Ref: 69-187)  
Taurine: 53 mc mol/L (Ref: 16-170)  
Threonine: 77 mc mol/L (Ref: 35-226)  
Tryptophan: 48 mc mol/L (Ref: 31-79)  
Tyrosine: 46 mc mol/L (Ref: 24-115)  
Valine: 189 mc mol/L (Ref: 74-321)  

Other Amino Acid: free homocystine = 0 mc mol/L
Interpretation: Timothy is an 18 month old male being seen to evaluate signs of failure to thrive. The urine amino acid concentrations are normalized to creatinine. The patient’s low creatinine of 29 mg/dL (RI 40-200) may have falsely elevated several amino acids. Interpret results with caution.

URINE AMINO ACIDS:
U 1 Methylhistidine: 614 mc mol/gm Cr (Ref: 1-980)
U 3 Methylhistidine: 528 mc mol/gm Cr (Ref: 20-610)
U Alanine: 1266 mc mol/gm Cr (Ref: 292-1151)
U alpha Aminobutyric Acid: 124 mc mol/gm Cr (Ref: 1-80)
U Aminoadipic Acid: 0 mc mol/gm Cr (Ref: 1-80)
U Arginine: 128 mc mol/gm Cr (Ref: 1-80)
U Asparagine: 507 mc mol/gm Cr (Ref: 1-283)
U Aspartic Acid: 55 mc mol/gm Cr (Ref: 18-89)
U Citrulline: 34 mc mol/gm Cr (Ref: 1-62)
U Cystine: 214 mc mol/gm Cr (Ref: 53-186)
U gamma Aminobutyric Acid: 38 mc mol/gm Cr (Ref: 24-262)
U Glutamic Acid: 252 mc mol/gm Cr (Ref: 1-97)
U Glutamine: 2852 mc mol/gm Cr (Ref: 308-2080)
U Glycine: 6348 mc mol/gm Cr (Ref: 974-3151)
U Histidine: 4052 mc mol/gm Cr (Ref: 602-2540)
U Homocysteine: 0 mc mol/gm Cr (Ref: c=1)
U Hydroxypro: 138 mc mol/gm Cr (Ref: 1-115)
U Isoleucine: 52 mc mol/gm Cr (Ref: 1-53)
U Leucine: 124 mc mol/gm Cr (Ref: 27-159)
U Lysine: 624 mc mol/gm Cr (Ref: 89-611)
U Methionine: 66 mc mol/gm Cr (Ref: 44-257)
U Ornithine: 72 mc mol/gm Cr (Ref: 1-71)
U Phenylalanine: 231 mc mol/gm Cr (Ref: 62-274)
U Proline: 100 mc mol/gm Cr (Ref: 1-80)
U Serine: 2224 mc mol/gm Cr (Ref: 283-1097)
U Taurine: 1803 mc mol/gm Cr (Ref: 106-1770)
U Threonine: 1369 mc mol/gm Cr (Ref: 89-549)
U Tyrosine: 510 mc mol/gm Cr (Ref: 89-425)
U Valine: 214 mc mol/gm Cr (Ref: 1-71)

Ur Creatinine: 29 mg/dL (Ref: 40-200)

Identifying Information/Interval History/Parent Concerns:
Timothy presents to the clinic today with mother and father for a well visit. Chart reviewed and history obtained from parents. He is currently under treatment with multiple specialists for severe failure to thrive, rickets and developmental delay. He has had initial outpatient follow up visits with endocrine, GI, nutrition, feeding therapy, ortho, pulmonary, PT and OT. Initial neuro and ophtho consults scheduled. He has TeiS and therapy in the works. We are attempting to schedule genetics follow up. His urine amino acids were abnormal but possible invalidated by a low urine creatinine. He has sweat chloride testing scheduled. Parents report he will now scoot on his bottom and he is starting to pull to a standing position. He does not get to a sitting position from lying nor crawl yet. His vocabulary is increasing and he knows > 10 words. He has responded well to VITD and his daily vit D was stopped at his endo visit and calcium decreased. He is due for repeat 2 wk f/u endo labs today. At nutrition visit last week parents were instructed to add 2 bolus feeds during the day but mom has not started these yet. He recently took Clinda for cervical lymphadenitis, this is much improved.
Other Chemistry DMTs

- Inborn errors of metabolism
  - Plasma AA, organic acids, acylcarnitine, genetic analysis
- Volatile ingestion
  - Osmolality, GC/HS, urinalysis/crystals, lytes, pH, osmolal gap
- Miscellaneous tox (Poison control center/ED)
  - Pain management
  - Acid/base MS with additional reflexive testing as needed for unknown ingestions
  - Newer designer drugs of abuse
- Endocrine
  - Aldosterone/renin ratio for refractory hypertension- screen for adrenal adenoma
  - CAH panel – testosterone, androstenedione, cortisol, 17-OH pregnenalone, progesterone, 17-OH progesterone, testosterone
The Dilemma of Advancing Science

➢ Rapidly advancing laboratory technologies offer opportunity to deliver on promise of personalized medicine to provide tailored care for each individual across the lifespan

➢ Evidence demonstrating improved patient outcomes from these technologies often lags behind adoption

➢ Escalating costs of innovative tests (up to 14% increase) is outpacing rising health-care costs (4-5% per year) with some genetic tests exceeding $10,000 for a patient

➢ VUMC chartered a Laboratory Formulary Committee to control laboratory costs patterned after the Pharmacy Formulary Committee that controls cost of medication
The Laboratory Formulary Committee

▪ Responsible for all inpatient and outpatient lab ordering
▪ Reviews evidence, consults with content experts and develops guidelines, substitutions, or restrictions to promote appropriate utilization of laboratory testing
▪ Subcommittee of Pharmacy, Therapeutics and Diagnostics Committee – exists outside any department and comprises voting members from diverse clinical departments and specialties with only a single pathologist
▪ Assisted by lab medical directors, administrators, finance representatives, LIS specialists, IT developers, data analysts, genetic counselors and project managers
Laboratory Formulary Examples

- Lab formulary focused on eliminating tests that were no longer gold standard or were of limited clinical utility
- Fractionated 25-OH vitamin D, only recommended when total vitamin D levels do not align with clinical presentation, but was often being ordered together with total Vitamin D = cost savings $102,842.

Fractionated 25-Hydroxyvitamin-D Orders Before and After Orderable Elimination

The ability to generate new orders for fractionated 25-hydroxyvitamin D was removed from the electronic ordering system in August 2015 (dotted line). The blue line represents total orders per month before and after the orderable removal.

Source: Authors
NEJM Catalyst (catalyst.nejm.org) © Massachusetts Medical Society
Laboratory Formulary Examples

• TAT for esoteric test results often exceed length of stay.
• Lab formulary required tests with median TAT > 7 days to be approved by on-call medical director before inpatient ordering – restriction decreased orders by 48% = **cost savings $257,745**
• Expert review of genetic tests to evaluate appropriateness of orders given clinical presentation, price variations among labs, optimal reflex testing and best TATs.
• LIS system implemented to facilitate holding samples for review and quickly redirecting to new labs without need for new blood draws. Genetic test review = **$394, 625 cost savings**
Summary

✓ Medical knowledge is growing exponentially
✓ Challenge for physicians to stay abreast of latest laboratory testing and algorithms
✓ DMTs assist physicians by providing expert interpretative guidance towards best practice and support in answering diagnostic questions
✓ Opportunity for the laboratory to actively participate on the healthcare team

Thanks to Dr. Michael Laposata for use of some introductory slides for this presentation
Thank You!