AN INTRODUCTION TO THE ROLE AND USE OF PATHOLOGY LABORATORY TESTING IN CLINICAL PRACTICE

Dr Tom Hartley,
RHH Pathology Services & UTas HLS

March 2013

CAM 201
SETTING THE SCENE: DIAGNOSTIC SERVICES

- Diagnostic Services – where does the Pathology Laboratory fit in?

- Group One: Medical “Imaging” … X-Ray, CAT scan, Ultrasound, Angiography, Endoscopy, NMR, PET, Nuclear Medicine ….

- Group Two: Pathology “Testing”: 5 Main Disciplines across 13 Laboratories
  - Anatomical Pathology
  - Clinical Biochemistry
  - Coagulation
  - Cytogenetics
  - Cytology
  - Endocrinology
  - Haematology
  - Infection Control
  - Microbiology
  - Molecular Medicine
  - Phlebotomy Service
  - Post Mortem
  - Transfusion Medicine
TEXTBOOKS

You can’t get by without one ..

http://www.labtestbook.com/index.html

$36.99 $18.41
Save $18.58 (50%) with Free Shipping!
Pathology Decision Support Tools by Alphabetical Order

A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z

-  
-***** PDST Example *****-  

A  
Anaphylaxis  
Antenatal Screening  
Arthritis  

B  
Bone pain in adults  
Bowel Cancer Screening
? Pituitary Disease or Tumour

Patient with headaches, visual disturbances, pituitary hyper- or hypo-secretion, or incidental finding on imaging

Careful clinical history

Physical examination including visual field assessment and review of past photos for comparison

Initial screening:
- Prolactin (if raised, exclude macroprolactin)
- TFTs: fT4 & TSH [1]
- Morning testosterone and LH in males
- FSH and oestradiol in postmenopausal women
- Assess menstrual history in young women
- U&E

Hormonal hypersecretion
- Suspicious for Cushing's syndrome
  - Overnight 1 mg dexamethasone suppression (not for women on oestrogen)
  - midnight salivary cortisol
  - 24-hour urinary free cortisol

Hormonal hypossecretion
- Suspicious for acromegaly
- Suspicious for ACTH deficiency (AD)
  - Serum 8-9 am cortisol
    - <100
    - 100-400
    - >400
  - IGF-1, GH
    - AD likely
    - AD equivocal
    - AD unlikely
- Suspicious for diabetes insipidus
  - U&E
  - Simultaneous plasma and urine osmolalities
  - Overnight water deprivation: fasting plasma and urine osmolalities

Consider synacthen stimulation test

Refer to endocrinologist for review; MRI pituitary
**Specimen:** Serum (preferred) or plasma

**Volume:** 1 mL

**Minimum Volume:** 0.5 mL

**Container:** Red-top tube, gel-barrier tube, green-top (heparin) tube, or lavender-top (EDTA) tube

**Collection:** Separate serum or plasma from cells within 45 minutes of collection.

**Storage Instructions:** Maintain specimen at room temperature.

**Stability:**
- **Temperature** | **Period**
  - Room temperature | 14 days
  - Refrigerated | 14 days
  - Frozen | 14 days
  - Freeze/thaw cycles stable x3

**Causes for Rejection:** Improper labeling

**Reference Interval:**

<table>
<thead>
<tr>
<th>Age</th>
<th>Acceptable</th>
<th>Borderline</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–19 years</td>
<td>&lt;170 (or 100–169)</td>
<td>170–199</td>
<td>≥200</td>
</tr>
<tr>
<td>20–24 years</td>
<td>&lt;190 (or 100–189)</td>
<td>190–224</td>
<td>≥225</td>
</tr>
<tr>
<td>&gt;24 years</td>
<td>&lt;200 (or 100–199)</td>
<td>200–239</td>
<td>≥240</td>
</tr>
</tbody>
</table>

**Use:** Evaluate lipid status and metabolic disorders. High levels of cholesterol that reflect high levels of HPLs may be caused by an inherited defect in lipoprotein metabolism, by disease of the endocrine system, by liver disease, or by renal disease. Low levels of cholesterol in the plasma may reflect an inherited deficiency of either LDL or HDL, or they may reflect impairment of liver function. Various hormone conditions are also related to cholesterol levels. Increased serum cholesterol in hypothyroid persons shows an increased LDL and decreased HDL. Low cholesterol are found in cases of hyperthyroidism, severe liver disease, pernicious anemia, and with increased estrogens. Pregnancy is accompanied by a moderate increase. Cholesterol is increased in early hepatitis, obstructed bile ducts, primary biliary cirrhosis, nephrotic syndrome, and diabetic meningitis. Finally, through much controversy, it appears that cholesterol is implicated in atherosclerosis and heart disease. Evaluate risk of coronary arterial occlusion, atherosclerosis, myocardial infarction, and complications including the demise of the patient.
Australian & UK Labs Use SI Units

To Convert to SI units:

$$(\text{mg/dl} \times 10)$$

Molecular Weight

= mmol/L
Clinical Analyte Unit Conversion
(Requires JavaScript)

1. Select Analyte
2. Select Units
3. Enter number to be converted in Value box
4. Press Enter or click Calculate

**Analyte**

Cholesterol

**Convert from**

mg/dL

**Value**

145

**Convert to**

mmol/L

**Answer**

3.75

**Factor**

0.02586

[Calculate]

[Reset]

You can purchase your own executable version of this program which includes molecular structures, empirical formula and formula mass. Click on the image below to see a screenshot.
First question – what is the probability that this Test Result I have just got back from the Lab ...eg a serum albumin concentration ...... is abnormal?

To make this decision you need to know some basic probability and statistical theory .... And this puts a lot of people off thinking about their laboratory data critically
Conventionally laboratories report normal ranges that encompass the values for that test observed in 95% of healthy individuals.

So if we have a normal range for Serum Albumin 35 – 50 g/L then there is a 0.025 probability that a healthy person could have a serum albumin of less than 35. Equally there is a 0.025 probability that a healthy person could have a serum albumin of greater than 50.
The Statistician’s Normal Distribution Curve
The Clinician’s Normal Distribution Curve

Serum Albumin: Reference Interval = 35 – 50 g/L

Therefore: Mean = 42.5 and SD = 3.75 and 2.5% of normal patients have a serum albumin less than 35 g/L and 100% of normal patients have serum albumins between 31.25 g/L and 53.75 g/L
But not all Laboratory data are normally distributed eg Platelets

So Laboratories should use Parametric and Non-Parametric Reference Intervals as appropriate to the distribution of results from normal individuals.
Steps in getting a reliable Pathology Result

- Correct patient
- Correct patient preparation
- Correct Sample Container
- Correct Sampling Site
- Correct Sample Labelling
- Complete Pathology Request Form with Clinical Notes
- Correct Sample Storage and Transport
- Sent to the Correct Laboratory on the correct day
Second Question: How can you diagnose a disease from a laboratory result?

“How can I diagnose Gestational Diabetes from my patient’s fasting blood glucose?”
Figure 1. Distribution of negative and positive GDMs using fasting blood sugar testing under the receiving operating characteristics curve.
To answer this properly we need to talk about

- Test Specificity
- Test Sensitivity
IDEAL SEPARATION OF NORMALS FROM PATIENTS
TYPICAL OVERLAP OF RESULTS FROM NORMALS AND RESULTS FROM PATIENTS
What does this result from a new patient mean?
Specificity and Sensitivity of a Test

Specificity: the probability that a laboratory test will be negative in the absence of a disease

= \# of true negatives divided by (\# of true negative + \# of false positives)
True Neg

Diagnostic Criterion

SP = TN / (TN + FP)

NORMALS
Sensitivity: the probability that a laboratory test is positive in the presence of disease

= # of true positives divided by ( # of true positives and # of false negatives)
Diagnostic Criterion

$$\text{SEN} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$
We can use a ‘Receiver Operator Curve’, which is a plot of (1-Specificity) on the x axis vs Sensitivity on the y axis, to visualise what happens when we move the ‘diagnostic threshold’
ROC Simulation

![ROC Simulation Chart]

- **Value** axis ranges from 0 to 180.
- Two groups are compared:
  - **Normals 0 - 100**
  - **Diseased 60 - 160**

The chart visualizes the distribution of values for normals and diseased groups, indicating differences in the data.
<table>
<thead>
<tr>
<th>Threshold</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>70</th>
<th>75</th>
<th>80</th>
<th>85</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positives</td>
<td>100</td>
<td>99</td>
<td>99</td>
<td>97</td>
<td>96</td>
<td>94</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>False Positives</td>
<td>43</td>
<td>30</td>
<td>28</td>
<td>19</td>
<td>13</td>
<td>8</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>True Negatives</td>
<td>57</td>
<td>70</td>
<td>72</td>
<td>81</td>
<td>87</td>
<td>92</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>False Negatives</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>1 - Specificity</td>
<td>0.43</td>
<td>0.3</td>
<td>0.28</td>
<td>0.19</td>
<td>0.13</td>
<td>0.08</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1.0</td>
<td>0.99</td>
<td>0.99</td>
<td>0.97</td>
<td>0.96</td>
<td>0.94</td>
<td>0.89</td>
<td>0.8</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.57</td>
<td>0.7</td>
<td>0.72</td>
<td>0.81</td>
<td>0.87</td>
<td>0.92</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>Checksum</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>sum</td>
<td>1.57</td>
<td>1.69</td>
<td>1.71</td>
<td>1.78</td>
<td>1.83</td>
<td>1.86</td>
<td>1.86</td>
<td>1.79</td>
</tr>
</tbody>
</table>

**ROC Curve**

![ROC Curve](image)
Third Question: How do I know that there has been a Significant change in my patient’s Laboratory Result?

- When the difference between the previous result and today’s result is greater than 2.77 times the Standard Deviation of the Laboratory’s Analytical Method eg.

  01/12/07 Cholesterol = 5.65
  12/02/08 Cholesterol = 5.75
  Laboratory’s SD of their analysis = 0.17 mmol/L
  Difference = 0.10 ....
  Diff/SD = 0.10/0.17 = 0.59 NOT SIGNIFICANT!
  A significant difference would be +/- 0.47 mmol/L!
Fourth Question: Why do you need Pathology Tests??

To gather evidence to prove and/or disprove hypothetical diagnoses.

- This means you need hypotheses
- To make hypotheses you need to have a knowledge of the underlying physiology of your hypotheses
- The best way is to have a flow diagram of the pathophysiology which shows where you will START ‘pathology testing’ with decision points that show when you will STOP ‘pathology testing’ ..
HYponatREMIA

↓ serum Na⁺

Check serum osmolality

>290 mOsm/kg

Hypertonic hyponatremia

Consider:
Hyperglycemia
Mannitol
Maltose

275-290 mOsm/kg

Isotonic hyponatremia (pseudohyponatremia)

Consider:
Hyperlipidemia
Hyperproteinemia
Bladder irrigation

<275 mOsm/kg

Hypotonic hyponatremia

Assess volume status

See next page
HYPONATREMIA (continued)

Assess volume status

Hypovolemic

Check urine Na⁺

Urine Na⁺ < 20 mEq/L
- Vomiting
- Diarrhea
- Third-space loss
- Insensible loss

Urine Na⁺ > 20 mEq/L
- Diuretics
- Mineralocorticoid deficiency
- Salt-losing nephropathies
- Ketonuria
- Osmotic diuresis
- Bicarbonaturia
- Cerebral salt wasting syndrome

Check urine osmolality

Euvolemic

Hypervolemic

Check urine Na⁺

Urine Na⁺ < 20 mEq/L
- CHF, cirrhosis, nephrotic syndrome

Urine Na⁺ > 20 mEq/L
- ARF, CRF

>100 mOsm/kg

<100 mOsm/kg → primary polydipsia, beer drinkers, potomania, tea and toast diet

Consider medication

SIADH if the following criteria are met:

- Normal adrenal, renal, and thyroid function
- Normal acid-base status
- Urine Na⁺ > 40 mEq/L

↑TSH + ↓free T4 → hypothyroidism

Abnormal cosynotropin stimulation test → adrenal insufficiency
### Identifications

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>032295</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient ID</td>
<td></td>
</tr>
<tr>
<td>Patient Last Name</td>
<td></td>
</tr>
<tr>
<td>Patient First Name</td>
<td>Syma</td>
</tr>
<tr>
<td>$\text{FO}_2(\text{I})$</td>
<td>21.0 %</td>
</tr>
<tr>
<td>Sample type</td>
<td>Not specified</td>
</tr>
<tr>
<td>Operator</td>
<td></td>
</tr>
</tbody>
</table>

### Blood Gas Values

<table>
<thead>
<tr>
<th>pH</th>
<th>7.377</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p\text{CO}_2$</td>
<td>43.9 mmHg</td>
</tr>
<tr>
<td>$p\text{O}_2$</td>
<td>56.9 mmHg</td>
</tr>
<tr>
<td>$c\text{HCO}_3^-(\text{P})_c$</td>
<td>25.2 mmol/L</td>
</tr>
<tr>
<td>$c\text{Base}(\text{B})_c$</td>
<td>0.4 mmol/L</td>
</tr>
</tbody>
</table>

### Electrolyte Values

| $c\text{Na}^+$ | 141 mmol/L |
| $c\text{K}^+$  | 4.1 mmol/L |
| $c\text{Ca}^{2+}$ | 1.18 mmol/L |
| $c\text{Ca}^{2+}(7.4)_c$ | 1.16 mmol/L |
| $c\text{Cl}^-$  | 105 mmol/L |

### Metabolite Values

| $c\text{Lac}$  | 1.8 mmol/L |
| $c\text{Glu}$  | 6.0 mmol/L |

### Oximetry Values

| $s\text{O}_2$     | 86.6 % |
| $ct\text{Hb}$    | 110 g/L |
| $F\text{CO}_2\text{Hb}$ | 0.7 % |
| $F\text{MetHb}$  | 28.3 % |
| $F\text{O}_2\text{Hb}$ | 61.5 % |
Clinical Trials: The majority are heavily reliant upon carefully controlled pathology testing performed by carefully chosen pathology laboratories.

CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials

Kenneth F Schulz,1 Douglas G Altman,2 David Moher,3 for the CONSORT Group

BMJ 2010;340:c332
Flow diagram of the progress through the phases of a parallel randomised trial of two groups (that is, enrolment, intervention allocation, follow-up, and data analysis)
**Background:** It may be safe to omit additional diagnostic testing in selected patients with suspected pulmonary embolism (PE) who have a negative D-dimer test, but this approach has never been evaluated in a randomized, controlled trial.

**Objective:** To determine if additional diagnostic testing can be safely withheld in patients with suspected PE who have negative erythrocyte agglutination D-dimer test results.
Figure 1. Study flow diagram.

Assessed for eligibility (n = 2591)

Eligible (n = 1260)

Declined to participate (n = 134)

Enrolled (n = 1126)
  Low clinical probability: 670
  Moderate or high clinical probability: 456

Eligible for random assignment (n = 459)

Not randomly assigned (n = 3)
  Low clinical probability: 0
  Moderate or high clinical probability: 3

Randomly assigned (n = 456)

Assigned to control strategies (n = 227)
  Received assigned intervention: 224
  Scheduled testing not done: 3

Lost to follow-up (n = 1)
  Analyzed (n = 226)

Assigned to and received experimental strategy of no additional diagnostic testing (n = 229)

Lost to follow-up (n = 6)
  Analyzed (n = 223)

Received usual diagnostic testing and management (n = 669)

Lost to follow-up (n = 6)

Excluded (n = 1331)
  Previous lung scan or ultrasonography: 668
  Heparin therapy for > 24 h or long-term warfarin therapy: 356
  Physician decided patient was inappropriate for study: 149
  Expected survival < 3 mo: 79
  Contraindication to radiographic contrast: 70
  Inaccessible for follow-up: 61
  Pregnancy: 46
Conclusion: In patients with a low probability of PE who have negative \( \text{d-dimer} \) results, additional diagnostic testing can be withheld without increasing the frequency of VTE during follow-up. Low clinical probability and negative \( \text{d-dimer} \) results occur in 50% of outpatients and in 20% of inpatients with suspected PE.
Pathology Testing comes to the aid of the 21 most underdiagnosed diseases

http://www.wrongdiagnosis.com/

<table>
<thead>
<tr>
<th></th>
<th>Indicates conditions that are best diagnosed via pathology testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Type 2 diabetes and Impaired glucose tolerance ✓</td>
</tr>
<tr>
<td>2.</td>
<td>High cholesterol ✓</td>
</tr>
<tr>
<td>3.</td>
<td>Hypertension</td>
</tr>
<tr>
<td>4.</td>
<td>Osteoporosis ✓</td>
</tr>
<tr>
<td>5.</td>
<td>Sexually transmitted diseases ✓</td>
</tr>
<tr>
<td>6.</td>
<td>Hemochromatosis ✓</td>
</tr>
<tr>
<td>7.</td>
<td>Chronic kidney disease ✓</td>
</tr>
<tr>
<td>8.</td>
<td>Hypothyroidism including Hashimoto's thyroiditis ✓</td>
</tr>
<tr>
<td>9.</td>
<td>Glaucoma</td>
</tr>
<tr>
<td>10.</td>
<td>Depression</td>
</tr>
</tbody>
</table>
Pathology Testing comes to the aid of the 21 most underdiagnosed diseases

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11.</td>
<td>Infectious diarrhea ✓</td>
</tr>
<tr>
<td>12.</td>
<td>Fecal incontinence</td>
</tr>
<tr>
<td>13.</td>
<td>Lactose intolerance ✓</td>
</tr>
<tr>
<td>14.</td>
<td>Polycystic ovary syndrome (PCOS) ✓</td>
</tr>
<tr>
<td>15.</td>
<td>Flat feet</td>
</tr>
<tr>
<td>16.</td>
<td>Attention deficit hyperactivity disorder and hyperactivity</td>
</tr>
<tr>
<td>17.</td>
<td>Sleep disorders such as sleep apnoea ✓</td>
</tr>
<tr>
<td>18.</td>
<td>Asthma ✓</td>
</tr>
<tr>
<td>19.</td>
<td>Bipolar disorder</td>
</tr>
<tr>
<td>20.</td>
<td>Celiac disease ✓</td>
</tr>
<tr>
<td>21.</td>
<td>Whooping cough (or pertussis) ✓</td>
</tr>
</tbody>
</table>
SUMMARY

- Pathology testing is usually the most convenient ‘diagnostic’ for both patient and clinician.
- Results need to be interpreted ‘statistically’ viz how abnormal, how ‘big’ is the change, what is the specificity and sensitivity of the test in the patient’s ‘suspected’ condition.
- Testing protocol needs to be designed to match the ‘suspected’ pathophysiology and/or rule out other ‘pathologies’. Negative results are often just as valuable as positives.
- There are good online resources as well as good ‘pocket’ guides…. You will not be able to get by without one.
- Pathology results are only as good as the specimen.
- Pathology results are only “useful” if they have been requested because you have a diagnostic ‘hypothesis’ to prove or disprove.
- View this presentation and more on www.medlabstats.com/students
Lecture: AN INTRODUCTION TO THE ROLE AND USE OF PATHOLOGY LABORATORY TESTING IN CLINICAL PRACTICE: 2013

Background Reading: The CONSORT Statement

Background Reading: D-Dimer Testing and Pulmonary Embolus

Email comments and enquiries to tom.hartley@dhhs.tas.gov.au with the Subject = 'Lecture Notes: Pathology Testing'