GOAL

- This course aims to provide the optometrist with a better understanding of the uses, limitations and interpretations of laboratory tests commonly used in eye care.

Objectives

- Understand the indications for ordering specific laboratory investigations.
- Know the limitations of clinical laboratory testing as it is used in the optometric setting.
- Improve the ability to interpret laboratory results and apply them to ocular disease.

Methods of Incorporation into Optometric Practice

- Independent acquisition of lab work
- Suggest lab evaluations when making consultations
- Facilitate communications with healthcare practitioners

Advantages to Patient and Doctor

- Expedite initiation of therapy
- Guide consultation to subspecialists
- Enhances interaction/communication
- Eliminates visits to multiple doctors

Indications for Ordering Laboratory Tests

- Narrow differential diagnosis
- Monitoring disease activity
- Monitoring response to therapy
- Screening for occult disease
- Estimate prognosis
- Other:
  - Protect against malpractice
  - Educational purposes
Laboratory testing is NOT a substitute for a careful history and physical examination!

Shot-Gun approach to test selection is inefficient & not cost effective.

Sensitivity
- Percentage of people with a disease who have a positive test
- Sensitivity = TP/(TP + FN)

Specificity
- Percentage of disease free individuals with a negative test
- Specificity = TN/(TN + FP)

Predictive Value
- **Population Based
- Positive Predictive Value
  - Probability of disease given a positive test
  - PPV = TP/(TP + FP)
- Negative Predictive Value
  - Probability of non-disease given a negative test
  - NPV = TN/(TN + FN)

Blood Chemistry
- Enzymes
- Blood Sugar
- Lipids
- Vitamins
- Drug Levels
- Electrolytes
- Protein by Products
- Hormones
- Minerals
**Laboratory Tests - Normative Values**

- What defines normal
- Consider your patient population
  - Age and sex
  - Exercise, diet (fasting)
  - Medications, drugs
- Standardized values
  - Diabetes, lipids, PSA, INR

**Reference Ranges**

- Guidelines
- Specific to YOUR laboratory
- Consider:
  - Patient’s condition
  - Previous results
- 5% will be outside normal range *in the absence of disease*

**Blood Chemistry: Glucose Determination**

- Casual Plasma Glucose
- Fasting Plasma Glucose
- Post-Prandial Glucose
- Oral Glucose Tolerance Test

**Glucose Determination**

**Fasting Plasma Glucose (FPG)**

- NPO X at least 8 hours
- **American Diabetes Association (2013)**
  - Normal: < 100 mg/dl
  - Impaired Glucose Tolerance: 100 - 125 mg/dl
  - Diabetes (provisional): ≥ 126 mg/dl

- In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day.

**Oral Glucose Tolerance Test (OGTT)**

- “Glucose challenge to unmask defects”
- Used when highly suspicious of DM inspite of normal FPG & in pregnancy
- OGTT Protocol:
  - NPO x 10-16 hours
  - FPG
  - Glucose loading dose
  - Measure plasma glucose at 1/2, 1, 2, & 3 hours
Oral Glucose Tolerance Test (OGTT)

- Expected Values
  - 1/2 hour post loading: <200 mg/dl
  - 1 hour post loading: <200 mg/dl
  - 2 hours post loading: <140 mg/dl
  - 3 hours post loading: 70-115 mg/dl

- Special protocol for women & children
- High sensitivity but low specificity

Criticisms of OGTT

- Poorly reproducible & more costly
- Varies with preceding physical activity & diet
- Inconvenient to administer
- Unpleasant to patients
- Must be performed twice

Glycosylated Hemoglobin

- % total hemoglobin bound to glucose
- Directly proportional to amount of glucose available to the RBC over its 120 day life-span
- Index of long-term glucose control over the past 2-3 months
- Not affected by diet, exercise, drugs, etc.

Glycosylated Hemoglobin

- Normal (non-diabetic) 4-6%
- Diabetic >7%
  - good control/goal of tx < 7%
  - Higher % = poor control

- Hemoglobinopathies produce abnormal results

ADAG Study
- International, multicenter
- Correlate HbA1c & average glucose
- Results: HbA1c correlates highly with average glucose
  - \( eAG (mg/dl) = 28.7 \times HbA1c - 46.7 \)
  - Standardized assay

HbA1c Estimated Average Glucose (eAG)

<table>
<thead>
<tr>
<th>HbA1c (%)</th>
<th>eAG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>126</td>
</tr>
<tr>
<td>7</td>
<td>154</td>
</tr>
<tr>
<td>8</td>
<td>183</td>
</tr>
<tr>
<td>9</td>
<td>212</td>
</tr>
<tr>
<td>10</td>
<td>240</td>
</tr>
</tbody>
</table>
**Average Glucose**

- **Meter vs. eAG**
  - May be lower than average shown on meter
  - eAG based on 24 hour glucose level
  - Most people test more often when their glucose levels are low

**Case: Refractive Shift**

- 50 year old White Male
- **POHx**: IDDM without retinopathy, Mild Cataracts
- **PMHx**: COPD, IDDM x 2004, HL, HTN, EtOH abuse

**Refractions**

- **HABITUAL 2009**
  - OD: -1.25 DS 20/40
  - OS: -1.25 DS 20/40

- **MANIFEST 01/11**
  - OD: -0.25-0.25 x045 20/25+1
  - OS: Plano 20/25

- **MANIFEST 06/11**
  - OD: -0.25 -0.50 x075 20/20-3
  - OS: +0.75 -0.25 x105 20/25+2

**Diabetic Status**

- **Reported glucose values**
  - 2009: range: 26-500+
  - 01/2011: range: 60-300+
  - 06/2011: range: 90-250

- 2010: increase metformin and insulin

**Diagnostic Criteria for Diabetes Mellitus**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fasting Plasma Glucose</th>
<th>Glycosylated Hemoglobin</th>
<th>Oral Glucose Tolerance Test</th>
<th>Casual Plasma Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>≥ 126 mg/dl</td>
<td>≥ 6.5%</td>
<td>2hPG ≥ 200 mg/dl</td>
<td>≥ 200 mg/dl plus symptoms</td>
</tr>
<tr>
<td>Increased Risk for Diabetes</td>
<td>100-125 mg/dl</td>
<td>5.7 – 6.4%</td>
<td>2hPG 140-199 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>&lt; 100 mg/dl</td>
<td>&lt;5.7%</td>
<td>2hPG &lt; 140 mg/dl</td>
<td></td>
</tr>
</tbody>
</table>
Diagnostic Criteria for Diabetes Mellitus

- In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day.
- OGTT not recommended for routine clinical use
- HbA1c not used in:
  - Pregnancy, blood loss or transfusions, chronic kidney or liver disease, Fe deficient or B12 anemia, hemoglobinopathies

Case HG

- 78 year old AA male
- PMHs: NIDDM x age 44 (range: 98-202)
  - HTN
  - CKD
  - Carotid a. disease
  - Sickle cell trait
- POHx: unremarkable
- BVA: 20/25 OD, OS
- IOP: 15 OD, 16 OS
- Slit Lamp: unremarkable OU
- 2+ NS with peripheral cortical changes

Case HG Results

<table>
<thead>
<tr>
<th>GLYCOXYLATED Hb A1c</th>
<th>Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.9 H %</td>
<td>4.0 - 6.0</td>
</tr>
</tbody>
</table>

**INTERPRETATION FOR DIABETIC PATIENTS:**
1. Well controlled: 6.2 - 7.0 %.
2. Intermediate controlled: 7.0 - 9.0 %.
3. Poorly controlled: >9.0 %.

<table>
<thead>
<tr>
<th>SICKLE TEST</th>
<th>POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMOGLOBIN A1</td>
<td>60.3 L %</td>
</tr>
<tr>
<td>HEMOGLOBIN A2</td>
<td>2.1 L %</td>
</tr>
<tr>
<td>HEMOGLOBIN S</td>
<td>37.7 H %</td>
</tr>
</tbody>
</table>

HbA1c & Sickle Cell Trait

- Abnormal hemoglobin can affect A1c measurements
  - Premature RBC death
  - Depends on testing method**
    - Molecular charge vs. molecular structure
    - http://www.ngsp.org/interf.asp
HbA1c & Sickle Cell Trait

- Decreased exposure time leads to decreased %
- Identify the Hb variant
- Alternate testing methods
  - Mass spectrometry $$$
  - Fructosamine
  - Continuous glucose monitoring

What happens when the patient has sickle cell disease?

<table>
<thead>
<tr>
<th>Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLYCOXYLATED Hb A1c</td>
<td>dec %</td>
</tr>
</tbody>
</table>

Comment: No Hgb A1c present
- unable to monitor diabetes with Hgb A1c

Blood Chemistry: Lipid Determinations

- Total Serum Cholesterol
- High Density Lipoprotein Cholesterol (HDL)
- Triglycerides

Plasma Lipids

- Cholesterol
- Triglycerides
- Phospholipids
- Free Fatty Acids

Lipoproteins

- Lipids (hydrophobic) + Proteins (hydrophilic)

  - VLDL = very low density lipoproteins
    - mostly triglycerides
  - LDL = low density lipoproteins
    - mostly cholesterol
  - HDL = high density lipoproteins
    - mostly protein
  - Chylomicrons = mostly triglycerides
Total Serum Cholesterol (TC)

- Total of cholesterol in all types of lipoproteins
- NPO x 10-16 hours
- **Current Guidelines** (ATP III - 2004)
  - Adults >20 years of age
  - Desirable ≤ 200 mg/dl

**Awaiting new guidelines**

High Density Lipoprotein Cholesterol (HDL-C)

- NPO x 10-16 hours
- Isolated by centrifugation after VLDL & LDL are precipitated out
- Reference ranges:
  - < 40 mg/dL: low; major risk for heart disease
  - 40-59 mg/dL: “average/normal”
  - >60 mg/dL: high; protective against heart disease

Low Density Lipoprotein Cholesterol (LDL-C)

- **Calculated Value**
  - LDL-C = TC - HDL-C - (TG/5)
- **Reference Ranges**
  - < 100 mg/dL: Optimal
  - 100-129 mg/dL: Near/Above Optimal
  - 130-159 mg/dL: Borderline High
  - 160-189 mg/dL: High
  - 190 mg/dL: Very High

Triglycerides

- Reflects VLDL (no chylomicrons if fasting)
- Screening test for hyperlipidemia
- Appearance of refrigerated serum
  - turbid
  - creamy layer on top
- **Expected values**:
  - < 150 mg/dL: Normal
  - 150-199 mg/dL: Borderline high
  - 200-499 mg/dL: High
  - > 500 mg/dL: Very High

Hollenhorst Plaque

Major Coronary Artery Disease Risk Factors

- elevated LDL-cholesterol
- male sex
- family history of premature heart disease
- cigarette smoking
- hypertension
- low HDL-cholesterol
- diabetes mellitus
- history of CVA or PVD
- severe obesity
Predicting Risk of Coronary Artery Disease

- Higher LDL & lower HDL = Greater Risk
- “Desirable” total cholesterol level puts you at lower risk
- [www.americanheart.org](http://www.americanheart.org)
- 10-year CVD Risk Calculator

Blood Chemistry

Thyroid Function Tests

![Thyroid Flow Chart](image1)

- Hypothalamus
  - Thyrotropin Releasing Hormone (TRH)
- Anterior Pituitary
  - Thyroid Stimulating Hormone (TSH)
- Negative Feedback Loop
  - \( T_3 & T_4 \)
  - \( \uparrow \)
  - \( T_3 & T_4 \)
  - \( \downarrow \)
  - \( TSH \)
- Thyroid Gland
  - \( T_1 & T_4 \)

Thyroid Hormone Activity

- Free Hormones = biologically active
- Bound Hormones = biologically inactive
- Binding Protein = thyroid binding globulin (TBG)

Thyroid Function Tests

- Serum \( T_3 \) & \( T_4 \) Assays
- \( T_3 \) Resin Uptake (T3RU)
- Serum Thyrotropin Assay (TSH)
- Free \( T_4 \) Assay

Thyroid Screening Protocol

- Very, very sensitive
- **TSH Assay**
  - more sensitive test
  - 3rd generation or higher
- **Free \( T_4 \) Assay**
  - indirect measurement by immunoassay
Thyroid Screening Protocol

- TSH alone
  - if normal, rules out thyroid dysfunction
  - if abnormal, Free $T_4$ assay is performed

Thyroid Testing Cautions

- Assumes absence of pituitary & hypothalamic disease
- Not accurate in acutely ill patients
- Rheumatoid factor & other autoantibodies can produce falsely increased results
- Drugs can affect results
- Less than ideal agreement between different assays

Thyroid Function Labs Beyond the Typical

- Anti-thyroid Antibodies
  - Autoimmune thyroiditis
  - Grave’s disease & Hashimoto’s Thyroiditis
- Calcitonin
- Thyroglobulin
  - Used to evaluate eradication of thyroid tissue after treatment of thyroid carcinoma

Blood Chemistry Tests for Sarcoidosis
**Tests for Sarcoidosis**

- Non-specific
- Released by granulomas
  - Serum Lysozyme
  - Angiotensin Converting Enzyme (ACE)
    - Steroids inhibit ACE activity
    - Reflects severity of the disease
    - Monitors disease activity/response to tx
- Other
  - Chest x-ray, gallium scan, biopsy

**ACE & Serum Lysozyme**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>84%</td>
<td>94%</td>
<td>47%</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>60%</td>
<td>76%</td>
<td>12%</td>
</tr>
<tr>
<td>ACE &amp; Lysozyme</td>
<td></td>
<td></td>
<td><strong>83%</strong></td>
</tr>
</tbody>
</table>

**Blood Chemistry**

**Potassium**

- Electrolyte
- Functions
  - nerve conduction
  - muscle function
  - acid-base balance
- Hypokalemia = deficiency of K⁺
- Hyperkalemia = excess K⁺

**BUN & Creatinine**

- Waste products of protein metabolism
- Increased levels suggest kidney disease
- Need to measure kidney function when using contrast medium with CT or MRI

**C-Reactive Protein**

- Non-specific!
- Produced by the liver
  - Acute phase response to
    - Inflammation, Infection & Tissue damage
- Directly reflects intensity of underlying pathological process(es)
- Falls rapidly when stimulus ceases
- 0-1 mg/dl normal
- 1-10 mg/dl moderate elevation
- > 10 mg/dl marked elevation
C-Reactive Protein

- Activates complement pathway
- Strongly correlated with BMI
  - Decreases with weight loss
- BCP & HRT cause elevation
- Exercise & EtOH use cause decrease
- Decreases with statin use independent of effects on lipid profile

CRP & Cardiovascular Disease

- Strongly predicts future coronary events, stroke & peripheral arterial disease
  - Atherosclerosis & atherothrombotic events are inflammatory processes
  - Not a marker for the degree of atherosclerosis
  - May also contribute to pathogenesis

CRP & Cardiovascular Disease

- Following myocardial infarction
  - Reflects the level of myocardial necrosis
  - @ 48hrs values predict outcome

C-Reactive Protein

- Very high sensitivity for GCA
- Together with high ESR may be best predictor of GCA

Hematology

- Complete Blood Count (CBC)
- Tests for hemoglobinopathies
- Tests of coagulation/hemostasis
Complete Blood Count with Differential

- White blood cell count (WBC)
- Red blood cell count (RBC)
- Hematocrit (HCT)
- Hemoglobin (Hgb)
- Red Blood Cell Indices
  - Mean cell volume (MCV)
  - Mean cell hemoglobin (MCH)
  - Mean cell hemoglobin concentration (MCHC)
- Platelet count

RBC - Erythrocytes

- RBC Count - # of RBCs/mm³
- Hemoglobin - capacity to carry O₂ & CO₂
- Hematocrit
  - Height of RBC column after centrifugation
  - Expressed as a % of column of whole blood
- RBC Indices - calculated values

RBC Count

- Slightly higher in males
- Polycythemia = increased # of RBCs
  - causes hyperviscosity
- Size, shape & structure

RBC Indices

- Mean Corpuscular Volume (MCV)
  - Divide hematocrit by RBC count
  - Microcytosis = decreased MCV
  - Macrocytosis = increased MCV
- Mean Corpuscular Hemoglobin (MCH)
  - divide blood hemoglobin by RBC count
- Mean Corpuscular Hemoglobin Concentration (MCHC)
  - divide hemoglobin by hematocrit
  - average concentration of hemoglobin per cell

Differential Diagnosis of Anemia in Adults

Microcytic Anemia (Low MCV)
- Iron deficiency
- Thalassemia
- Chronic disease
- Sideroblastic (congenital, lead, drugs)
- Uncommon
- Copper deficiency, zinc poisoning - rare

Normocytic Anemia (Normal MCV)
- Acute blood loss
- Early iron deficiency
- Bone marrow suppression
- Chronic renal insufficiency
- Hypothyroidism
- Hypoplasmatism

Macrocytic Anemia (High MCV)
- Alcohol abuse
- Folic acid deficiency
- Vitamin B12 deficiency
- Acute myeloid leukemia
- Hemolytic anemia
- Drug toxicity
- Liver disease

White Blood Cell Count

- WBC (leukocyte) - 1st line of defense against infection
- Leukocytosis = high WBC count
  - pain, stress, exercise, pregnancy, cancer, MI, infection
- Leukopenia = low WBC count
  - exposure to ionizing radiation, viral illness, impaired immune system, bone marrow problems
**White Blood Cell Differential**

- Helps determine cause of abnormal hematologic state
- Types of WBCs
  - Granulocytes
    - Polymorphonuclear neutrophils
    - Polymorphonuclear eosinophils
    - Polymorphonuclear basophils
  - Non-Granulocytes
    - Monocytes
    - Lymphocytes
    - Plasma cells

**Neutrophilia**

- Increased neutrophil count
- Potential causes include:
  - Invading organisms (usually bacterial)
  - Tissue breakdown (burns, surgery, MI, cancer)
  - Drugs
  - Metabolic/toxic states (diabetic acidosis)
  - Hematologic disorders
  - Physiologic stressors

**Neutropenia**

- Decreased neutrophil count
- Potential causes include:
  - Deficient production (drugs, hypersplenism, aplastic anemia, etc.)
  - Depletion due to overwhelming bacterial infection (septicemia)

**Monocytosis**

- Increased number of monocytes
- Potential causes include:
  - Viral infection
  - Bacterial infection
  - Parasitic infection
  - Lymphoma
  - Multiple myeloma

**Lymphocytosis**

- Increased number of lymphocytes
- Most common cause: Viral infection
  - Relative lymphocytosis due to decreased granulocyte count
- Potential causes of absolute lymphocytosis:
  - Pertussis
  - Infectious lymphocytosis
  - Lymphocytic leukemia
  - Infectious mononucleosis
  - Adult cytomegalovirus infection

**Lymphopenia**

- Decreased number of lymphocytes
- Potential causes include:
  - HIV infection
  - Malignancy
  - Collagen vascular diseases
  - Radiation therapy
Eosinophilia

- Increased number of eosinophils
- Potential causes:
  - parasitic infection
  - allergy

Basophilia

- Increased basophil count
- Potential causes include:
  - myeloproliferative disorders
  - chronic myelogenous leukemia

---

**Case HJ 52 y.o. AA Male**

- Recalcitrant low grade nongranulomatous iritis OD
- PMHx: unremarkable
- BVA: 20/20 OD, OS
- Slit Lamp OD: 1+ cell

---

**Case HJ: Lab Results**

<table>
<thead>
<tr>
<th>Blood Parameter</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT</td>
<td>190 K/ul</td>
<td>130 - 400 K/ul</td>
</tr>
<tr>
<td>WBC</td>
<td>12.5 H K/uL</td>
<td>4 - 11 K/uL</td>
</tr>
<tr>
<td>RBC</td>
<td>3.88 L M/ul</td>
<td>4.2 - 5.7 M/ul</td>
</tr>
<tr>
<td>HGB</td>
<td>12.3 L g/dl</td>
<td>13 - 17 g/dl</td>
</tr>
<tr>
<td>HCT</td>
<td>37.2 L %</td>
<td>40 - 51 %</td>
</tr>
<tr>
<td>MCV</td>
<td>96.0 H fl</td>
<td>82 - 99 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>31.7 H pg</td>
<td>27 - 34 pg</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.0 g/dl</td>
<td>31 - 37 g/dl</td>
</tr>
<tr>
<td>RDW</td>
<td>14.2 %</td>
<td>&lt;15.0 %</td>
</tr>
<tr>
<td>MPV</td>
<td>8.0 fl</td>
<td>8 – 12 fl</td>
</tr>
</tbody>
</table>

---

**Case HJ: Lab Results**

<table>
<thead>
<tr>
<th>Blood Parameter</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEUTROPHILS</td>
<td>13 L %</td>
<td>40 - 80 %</td>
</tr>
<tr>
<td>LYMPH</td>
<td>81 H %</td>
<td>15 - 45 %</td>
</tr>
<tr>
<td>MONOS</td>
<td>4 %</td>
<td>2 – 12 %</td>
</tr>
<tr>
<td>EOSINOPHILS</td>
<td>2 %</td>
<td>0 - 6 %</td>
</tr>
</tbody>
</table>

**Note:** Persistent with repeated measurements
**Case HJ – Lab Results**

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Protein Electrophoresis (SPEP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>109</td>
<td>mg/dL</td>
</tr>
<tr>
<td>IgG</td>
<td>2595 H</td>
<td>mg/dL</td>
</tr>
<tr>
<td>IgM</td>
<td>71.1</td>
<td>mg/dL</td>
</tr>
<tr>
<td>KAPPA</td>
<td>2636 H</td>
<td>mg/dL</td>
</tr>
<tr>
<td>LAMBDA</td>
<td>232 L</td>
<td>mg/dL</td>
</tr>
<tr>
<td>RPR</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>FTA</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>HIV</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>20</td>
<td>U/L</td>
</tr>
<tr>
<td>LYSOZYME, SERUM</td>
<td>4.40</td>
<td>mcg/mL</td>
</tr>
<tr>
<td>ANA</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

**Case HJ**

- Urine: negative Bence Jones Protein
- Monoclonal Gammopathy of Unspecified Significance (MGUS)
- Iritis = Masquerade Syndrome

**Platelets - Thrombocytes**

- Function to activate blood clotting mechanism
- **Thrombocytopenia** = decreased # of platelets
  - Causes: immunologic, drug induced, idiopathic, post-transfusion, hypersplenism, bone marrow deficiency
- **Thrombocytosis** = increased # of platelets
  - Causes: malignancy, acute bleeding, acute infection, post-splenectomy, collagen vascular disease, myeloproliferative disorders

**Hematology**

**Erythrocyte Sedimentation Rate (ESR)**

- Millimeters of settled RBCs in 1 hour
- Non-specific indicator of increased immunoglobulin & fibrinogen levels
- Causes include: pregnancy, malignancy, injury, Giant Cell Arteritis
  - 99% sensitive
  - 70% specific

**ESR Reference Ranges**

- Age corrected
  - Men: Age/2
  - Women: (Age + 10)/2
Don’t forget to include GCA in DDx!

Prothrombin Time (PT)
- Assesses the extrinsic pathway of clotting
- Endpoint = time (in seconds) for the formation of a fibrin clot
- Affected by:
  - Vitamin K deficiency
  - Liver disease
  - Deficiency or inhibition of factors VII, X, II, V, or fibrinogen

International Normalized Ratio (INR)
- Developed by the WHO
- Promote standardization of PT for monitoring oral anticoagulant therapy
- Can be directly compared across different labs
  - INR = [Patient PT / Control PT]^{ISI}
    - ISI (international sensitivity index) is determined for each PT reagent and instrument combination
- Therapeutic range is 2.0 to 3.0
**Hematology**

**Tests for Hemoglobinopathies**

- Sickle Cell Trait (AS)
- Sickle Cell Anemia (SS)
- Sickle Cell C Disease (SC)
- Sickle Cell C Trait (AC)
- Thalessemias

**Sickling Test**

- Sickle Dex, Sickle Prep
- Reducing agent causes sickling if > 10% HbS
- Screening test

**Hemoglobin Electrophoresis**

- Hemolyzed RBCs on cellulose acetate separated by current
- Definitive test

**PDR in patient on Coumadin for Atrial Fibrillation**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin Time</td>
<td>38.1 H</td>
<td>9.5 - 11.7 sec</td>
</tr>
<tr>
<td>INR</td>
<td>3.76 H</td>
<td>0.9 - 1.1</td>
</tr>
</tbody>
</table>
Serology

Techniques of Serologic Testing
- Flocculation
- Immunofluorescent assay (IFA)
- Enzyme-Linked Immunosorbent Assay (ELISA)
- Western Blot
- Polymerase Chain Reaction (PCR)

Immunofluorescent Assay (IFA)

1. Serum & fixed organisms are combined

2. If present, antigen-specific antibodies bind to the fixed organisms, forming antigen-antibody complexes

3. Fluorescent tagged antiglobulin is added & binds to the Ag/Ab complexes

4. The fluorescent tagged Ag/Ab complexes are examined with a fluorescent microscope
ELISA:
The enzyme-linked antiglobulin reacts with the Ag-Ab complexes, activating the enzyme. The enzyme activity (color change) is then quantified.

Serology Test for Syphilis

- **Non-Treponemal (reagin) Tests**
  - Rapid Plasma Reagin (RPR)
  - Venereal Disease Research Laboratory (VDRL)
- **Treponemal Specific Tests**
  - Fluorescent Treponemal antibody Absorption Test (FTA-Abs)
  - Micro-Hemagglutination Treponemal Pallidum Test (MHA-TP)
  - *T. pallidum* enzyme immunoassay (EIA)

Rapid Plasma Reagin (RPR)

- Reagin = non-specific syphilis associated antibody
- Multiple antigens: cardiolipin, lecithin, cholesterol
- Become + ~ 6 weeks after exposure
- Low specificity

Rapid Plasma Reagin (RPR)

- **Biologic False Positives:**
  - infectious mononucleosis, malaria, pregnancy, lupus, rheumatoid arthritis, HIV, TB, IV drug use, bacterial endocarditis, disorders of Ig production

Rapid Plasma Reagin (RPR)

- **Primary Syphilis:** + titer (4-6 weeks)
- **Secondary Syphilis:** +++ titer
  *almost 100% sensitive
- **Latent/Tertiary Syphilis:** decreasing titers/sensitivity
- **Adequate Tx:** - titer
**Non-Treponemal Specific Test**
**RPR & VDRL**
- 15-20% serofast
  - < 1:8 following treatment
- 4 fold change in titer or 2 dilutions is significant
- Successful serologic response longer in HIV patients
- High titers may not decrease for 12-24 months following treatment
- Titers wane over time even without treatment

**Fluorescent Treponemal Antibody Absorption Test**
**(FTA-Abs)**
- Immunofluorescent assay technique
- +/- cross-reactivity with Lyme disease
- Remains positive after treatment
- Highly sensitive & specific

**Fluorescent Treponemal Antibody Absorption Test**
**(FTA-Abs)**
- Primary Syphilis: Up to 10% false -
- Secondary Syphilis: Almost no false -
- Latent/Tertiary Syphilis: Almost no false -
- Adequate Tx: Remains positive

**Interpretation of Syphilis Tests**

<table>
<thead>
<tr>
<th>FTA-Abs</th>
<th>RPR</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Active syphilis</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>Adequately treated syphilis</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>Biologic false positive</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>No exposure or very recent exposure</td>
</tr>
</tbody>
</table>

**T. Pallidum EIA**
- Assesses IgG antibodies to syphilis
  - Detects in all stages
- Same or better sensitivity & specificity
- Objective, potential to be automated

**NEW Syphilis Testing Sequence**
- **T. pallidum EIA** first
  - If positive → qualitative/quantitative RPR
    - BOTH positive = diagnosis of syphilis, esp. if RPR ≥ 8
  - If negative → no further testing
    - Not syphilis or early syphilis
### Syphilis Testing Algorithm

- **Nonreactive**
  - **EIA**
    - **Reactive**
      - **RPR test**
        - **Nonreactive**
          - **FTA-Abs**
            - **Reactive**
              - **Syphilis**
        - **Reactive**
          - **Late latent or treated?**
            - **Nonreactive**
              - **Syphilis**
            - **Reactive**
              - **Syphilis**

Cases AT 66 year old AAM

- Bilateral optic atrophy OS>OD
- PMHx: Hepatitis C, COPD, ETOH/cocaine abuse, HTN, BPH
- BVA: 20/20 OD, OS
- Color (Ishihara): 10/13 OD, 9/13 OS
- Slit Lamp: unremarkable

### Case AT: Lab Results

<table>
<thead>
<tr>
<th>Specimen Collection Date</th>
<th>Test</th>
<th>Result 1</th>
<th>Reference Range</th>
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<tbody>
<tr>
<td>11/24/2006</td>
<td>MHATP</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
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<tr>
<td></td>
<td>RPR QUANTITATIVE</td>
<td>Reactive 1:16</td>
<td>Non-reactive</td>
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<td>01/05/2007</td>
<td>MHATP</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td></td>
<td>RPR</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>01/12/2007</td>
<td>MHATP</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td></td>
<td>RPR QUANTITATIVE</td>
<td>Reactive 1:8</td>
<td>Non-reactive</td>
</tr>
<tr>
<td></td>
<td>HIV</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

### Case AT

- Biologic False Positive RPR

### Lyme Disease
Serologic Testing for Lyme Disease

- IFA for IgM & IgG
- ELISA for IgM & IgG
  - more sensitive & more specific
- Up to 50% false negatives in early infection
- cross-reactivity with other spirochetes
- Positive ELISA confirmed by Western Blot

Lyme Disease: Special Considerations

1. Negative serology does NOT rule out infection
2. *B. burgdorferi* Ag may cross-react with FTA-Abs
3. *T. pallidum* Ag may cross-react with Lyme Serology
4. Treatment does not always result in seroconversion

Lyme Disease

Negative titers do NOT exclude the diagnosis!!

HIV

- Measurable Ab response in 6 - 10 weeks
- Latency up to 5 years
- ELISA for IgG & IgM
- Western Blot

Serologic Tests for HIV
HIV ELISA
- Tests for IgG & IgM
- Screening test
- Higher sensitivity, lower specificity
- Higher rate of false + in low risk groups
- Both HIV-1 and HIV-2 since 1992

HIV Western Blot
- Confirmatory test
- Higher specificity, lower sensitivity
- Positive predictive value of ELISA & Western Blot sequence in both high & low risk groups > 99%

HIV Infection

Special Considerations in Laboratory Testing
1. Decreased or absent immune response
   - Insufficient Ab production (ex. false - FTA-Abs)
   - Inadequate B-cell response (ex. false - PPD)
2. Polyclonal B-cell activation
   - ex. higher than normal titers, false + RPR
3. Multiple diseases frequently co-exist
   - ex. AIDS/TB/Syphilis/Lyme

HIV Staging
- CD4 lymphocyte count
- HIV plasma RNA (viral load)
- HIV enters host → Burst of viremia → Inhibited by onset of immune response → Equilibrium
- Production of 10 billion virons per day

CD4 Lymphocytes
- Predicts short-term risk of new opportunistic infections
- Reflects the effect of viral activity NOT the level of viral activity itself
  - degree of immune destruction
- < 200 cells/mm³ = diagnosis of AIDS
Plasma HIV RNA (Viral Load)

- Level of viral RNA in the blood
- PCR or Branched DNA methods
  - Can detect 1 million to <40 copies/ml (undetectable)
- A measure of virus production which drives CD4 lymphocyte destruction
  - Rate of CD4 destruction

HIV Viral Load

- Can not compare different testing methods
- Proper specimen handling is important
  - HIV RNA unstable in whole blood
- NOT approved as a diagnostic test
- Transient increase with:
  - Acute illness
  - Vaccination
  - Should return to baseline w/in one month
- http://aidsinfo.nih.gov/guidelines

Plasma HIV RNA Test Interpretation

- Predicts likelihood of developing AIDS (Prognosis)
  - Study by Mellors, et al. 1996
    - < 4,350 copies/ml: 8% AIDS in 5 yrs.
    - > 36,270 copies/ml: 62% AIDS in 5 yrs.
    - Better than CD4
- Monitors effectiveness of therapy
  - Decreases with antiviral treatment
  - 3-fold (0.5 log) change indicates a relevant change in viral replication

Plasma HIV RNA Recommendations

- 2 measurements (2-4 wks apart) at initial evaluation
- Every 3-4 months thereafter or as indicated by changes in therapy/clinical picture
- Interpreted in conjunction with CD4 count & patient’s clinical picture
- Ideal viral load is undetectable

MB 54 y.o. AA Male

- Inpatient: Admitted for chest pain
- Admission 1 month prior for candida esophagitis
- HIV+ noncompliant with HAART
- BVA: 20/30 OD, 20/40 OS
- IOP: 12 mmHg OD, OS
- Anterior segment unremarkable
### Specimen Collection Date: 07/06/2011

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<tr>
<th>Tests</th>
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<th>Reference Range</th>
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<td>CD3/CD4 ABS</td>
<td>13 L cells/μL 693 – 1319</td>
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<tr>
<td>HIV PCR QUANT.</td>
<td>147,000 copies/mL</td>
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### Specimen Collection Date: 11/04/2010

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<th>Results</th>
<th>Reference Range</th>
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</thead>
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<td>47 L cells/μL 693 – 1319</td>
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</tr>
<tr>
<td>HIV PCR QUANT.</td>
<td>112,000 copies/mL</td>
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</table>

---

**Case MH 40 y.o. W Male**

- **PMHx:** HIV+ x age 31
  - HL peripheral neuropathy
- **POHx:** OAG dry eye syndrome

---

**CD4 Count**

**Viral Load**
Case MH 40 y.o. W Male

- Drastic improvement in labs with introduction of HAART treatment

Case BB 45 y.o. W Male

- Right Homonymous Hemianopia
- Progressive multifocal leukoencephalopathy (PML)
- HIV/AIDS x 1993
  - Resistant virus
  - Multiple variations of HAART regimen
- Seizures
- Peripheral Neuropathy
- Cryptococcal Meningitis 2002
- Leukopenia/Pancytopenia 2003
- Necrotizing Pancreatitis 2004
- Deceased May 2004

Case BB

MRI - PML

Viral Load

- Immune system never recovered
  - Continued risk for opportunistic infection
John Coffin:

“The development of AIDS can be likened to an impending train wreck, where the viral load indicates the speed with which the train is headed for catastrophe & the CD4 cell count marks the distance from the site of doom. The means of slowing the train are now available, but ways of stopping & reversing the locomotive must be found.”

**Anti-Nuclear Antibody (ANA)**

- IFA technique
- Detects auto-antibodies against cellular nuclear material
- Positive titers with autoimmune diseases
- Not affected by steroid treatment

**ANA**

- Normal = titers < 1:20
- Patterns of fluorescence
  - Limited usefulness
    - Specific Ab assays
      - Anti-DNA, Sm, RNP, Ro/SSa or La/SSb
    - Speckled
    - Rim
    - Diffuse/Homogenous
    - Nucleolar
- Screening test
  - Automated ELISA method
  - Not as reliable

**Positive ANA in Autoimmune Diseases**

- Lupus (95%)
- Sjögren’s (40-85%)
- Scleroderma (60-90%)
- Mixed Connective Tissue Disease (93%)
- Myasthenia Gravis (30-55%)
- Juvenile chronic arthritis (22%)
- Rheumatoid Arthritis (20-40%)
- Over age 70 (10%)*

**Positive ANA**

- Specific Organ Autoimmune Diseases
  - Hashimoto’s Thyroiditis (46%)
  - Graves’ disease (50%)
  - Primary biliary cirrhosis (10-40%)
  - Primary autoimmune cholangitis
  - Idiopathic pulmonary arterial hypertension (40%)
- Others
  - Mononucleosis
  - Hepatitis C
  - Subacute bacterial endocarditis
  - Tuberculosis
  - HIV
**ANA**

- May be positive prior to signs/symptoms of disease
- False Positives
  - More common in women & elderly
  - 10-15% > 65 years
- Titers:
  - 1:40 32%
  - 1:80 13%
- **Higher titers = greater disease suspicion**

**Anti-Neutrophil Cytoplasmic Antibody (ANCA)**

- IgG antibodies against antigens in the cytoplasm of neutrophils
- ELISA or IFA technique
- Two types of antibodies:
  - p-ANCA - perinuclear staining pattern
  - C-ANCA – cytoplasmic staining pattern

**Rheumatoid Factor**

- Flocculation method
- Detects autoantibodies against IgG
- Associated with rheumatic conditions:
  - Rheumatoid Arthritis
  - Sjogren’s Syndrome
  - Lupus
  - Systemic Sclerosis
  - Polymyositis
Rheumatoid Factor

- Associated Non-Rheumatic Conditions:
  - Bacterial Endocarditis
  - Tuberculosis
  - Syphilis
  - Infectious Hepatitis
  - Sarcoidosis

- **Overall low sensitivity**

Case VD 49 y.o. AA Male

- c/o pain, photophobia, foreign body sensation and white discharge x 2 months
- PMHx: HTN
  - c/o non-specific arthralgia, fatigue and weakness
- BVA: 20/25 OD, 20/50 OS
- Slit Lamp:
  - Diffuse injection OS>OD
  - Numerous filaments & PEE OS>OD involving the visual axis
- TBUT: instantaneous
- Basal Schirmer: 1mm @ 5 minutes

Case VD: Lab Results

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>Reference Range</th>
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<tbody>
<tr>
<td>WESTERGREN*</td>
<td>45 H</td>
<td>0 - 15</td>
</tr>
<tr>
<td>RHEUMATOID FACTOR</td>
<td>130 H</td>
<td>0 - 15</td>
</tr>
<tr>
<td>ANA</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>56 ug/dL</td>
<td>30-80</td>
</tr>
</tbody>
</table>

Case VD

- Advanced K Sicca with filamentary keratopathy OS>OD
- Rheumatology referral
- Diagnosed with Sjogren’s Syndrome

Anti-Phospholipid Antibodies

- Antibodies directed against phospholipid-binding plasma proteins
- Can lead to antiphospholipid syndrome (APS)
  - Venous or arterial thromboses
  - Pregnancy morbidity
  - Thrombocytopenia

Anti-Phospholipid Antibodies

- Detected by:
  - Lupus anticoagulant tests
  - Anti-cardiolipin antibody ELISA
  - Anti-β2 glycoprotein-1 ELISA
- Must be confirmed with repeat testing
Case AB 65 y.o. AA Male

- c/o red painful OS (8/10) for 2 days
- PMHx:
  - Discoid Lupus
  - Peripheral vascular disease
  - Gangrene of multiple fingers & toes
  - Cocaine & opioid dependence
  - Anemia
- BVA: 20/30 OD & OS
- Slit Lamp OS:
  - 2+ diffuse, deep injection
  - 1+ cell

Case AB: Lab Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>ANA</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>speckled</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320 titer</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>&lt;10 IU/mL</td>
<td>0 - 15</td>
</tr>
<tr>
<td>ACE</td>
<td>60 U/L</td>
<td>9 - 67</td>
</tr>
<tr>
<td>Anti-ss DNA Antibody</td>
<td>312 H U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>246.02 H IU</td>
<td>0 - 24</td>
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</table>

Anti-Phospholipid Ab Syndrome

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<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-cardiolipin IGG</td>
<td>&lt;10 GPL U/mL</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Anti-cardiolipin IGM</td>
<td>&lt;10 MPL U/mL</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Anti-cardiolipin IGA</td>
<td>28 H APL U/mL</td>
<td>0 - 15</td>
</tr>
<tr>
<td>Reference Range:</td>
<td>&lt;10 NORMAL</td>
<td>10-15 EQUIVOCAL</td>
</tr>
<tr>
<td></td>
<td>&gt;15 POSITIVE</td>
<td></td>
</tr>
</tbody>
</table>

Case AB - ANA over time

- Inflammatory Orbital Pseudotumor
  - Developed choroidal...
Toxoplasmosis

Serologic Tests for Toxoplasmosis
- Sabin-Feldman dye test = Gold standard
- IFA for IgG & IgM
  - 95% agreement with dye test
- ELISA
  - as sensitive/specific as dye test

Antibody Response to Infection with *Toxoplasma gondii*
- Seroconversion at 1 - 2 weeks
- Peak at 6 - 8 weeks
- Gradual decline over months - years
- Weakly + titers for years

Ocular Toxoplasmosis (Rothora, et al)
- 100% + IgG titers in clinically apparent ocular toxoplasmosis
- 58% + IgG titers in controls
- ***Negative titer may be more significant than a positive titer.***

Toxocariasis
- ELISA
- 1 - 6% of normals test positive
- * Any positive titer with the appropriate clinical picture is highly suggestive of infection
Polymerase Chain Reaction

PCR

- DNA and some RNA are amplified and copied
- Then known segments are matched.
- Can be done on any sample

- Viral Infections
  - CMV, HSV, HZV

Tuberculosis

Tuberculosis Trends 2012

- 9,945 cases in US - 3.2/100,000 persons
- 63% occurred in foreign-born persons
- Lifetime risk of active disease: 5-10% if test +

- Globally (WHO)
  - 8.6 million develop active TB disease annually
  - 1/3 of world pop thought to be latently infected
  - 1.3 million deaths from active TB

Risk Factors for Progression Infection → Active Disease

- HIV infection
- Infants & children < 5 years
- Immunosuppressive therapy
- Recent (w/in 2yrs) infection
- h/o untreated or inadequately treated active TB
- Silicosis, DM, CRF, Leukemia, lymphoma, Ca of head, neck or lungs
- Post gastrectomy or jejunoileal bypass
- Weight <90% of ideal
- Smokers & drug abusers

Interferon Gamma Release Assays (IGRA)

- QuantiFERON®- TB Gold (QFT)
- QuantiFERON®-TB Gold In-Tube (QFT-GIT)
- T-SPOT TB test (T-spot)

***Test results may not be interchangeable.
***Test results may fluctuate with serial testing.
IGRA Test Method

- Heparinized whole blood incubated with mixture of synthetic peptides & a control (saline)
  - 2 proteins present in M. tuberculosis
    - Early secretory antigenic target-6 (ESAT-6)
    - Culture filtrate protein 10 (CFP-10)
  - Absent from Bacille Calmette-Guérin (BCG) & other commonly encountered mycobacteria
- In sensitized patients → Interferon-γ is released in vitro by T-lymphocytes & detected by ELISA

IGRA Test Method

- Amount of Interferon-γ released in response to peptides is calculated
  - Peptides minus control (nil)
- Test interpretation varies by test method
- Cell-mediated immune response

QuantiFERON®- TB Gold

- Used in place of but NOT in addition to a PPD skin test
- Preferred for testing:
  - Past BCG vaccine
  - Unable to return for reading

QuantiFERON®- TB Gold

- Results reported as:
  - Positive
  - Negative
  - Indeterminate
  - Age <5yrs or >80yrs
  - Immunosuppression
  - Consider repeating test

QuantiFERON®- TB Gold

- Nil value
  - Determines if patient has a preexisting immune response that could cause a false +
  - Negative control
  - Must have value ≤ 8.0 IU/ml to be valid

- Mitogen
  - Control for correct specimen handling & patient immune status

QuantiFERON®- TB Gold

- TB Antigen
  - TB specific polypeptides

- Result calculated from these values using an FDA-approved algorithm
  - Positive: TB Antigen minus Nil ≥ 0.35 IU/ml
QuantiFERON®- Gold

Advantages
- More specific than PPD (selected proteins)
- NOT affected by prior BCG vaccine
- NO boosting phenomenon
  - 2 step testing is unnecessary
- Results available < 24 hours
  - NO second visit!
- Not subject to placement and reading biases

Disadvantages
- Negative results alone DO NOT exclude TB
- Blood must be incubated w/ antigens ≤ 12 hrs. after collection
- Unable to distinguish latent infection from active disease
- Impaired by low CD4 count in HIV patients
- Not for use in children < 5yrs
- Higher cost ~ $30-60

Skin Tests
- Tuberculosis (PPD)
- Histoplasmosis
- Anergy Battery

QuantiFERON®- TB Gold QFT-G

Advantages
- More specific than PPD (selected proteins)
- NOT affected by prior BCG vaccine
- NO boosting phenomenon
  - 2 step testing is unnecessary
- Results available < 24 hours
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- Not for use in children < 5yrs
- Higher cost ~ $30-60

Skin Tests
- Tuberculosis (PPD)
- Histoplasmosis
- Anergy Battery

Delayed Hypersensitivity
- Antigen injected intradermally
- Positive = development of induration
  *NOT erythema
- Negative = no exposure or anergy
Tuberculosis Skin Test

- Intradermal injection of purified protein derivative (PPD)
- Delayed hypersensitivity in vivo response develops within 6 weeks after infection
- Remains positive throughout life
  - except with very early treatment
- Test is read 48 - 72 hours after injection

PPD Test Interpretation

- Positive: ≥ 10 mm induration
- Equivocal: 5 - 9 mm induration
- Negative: < 5 mm induration

- HIV Infection:
  - Positive: ≥ 5 mm induration

Tuberculosis Skin Test

- False Negatives associated with anergy producing conditions:
  - Severe TB
  - Sarcoid
  - Debility
  - Increasing age
  - Steroid treatment
  - AIDS or other immunocompromise

Anergy

- AIDS, immunosuppression or sarcoidosis
- Anergy Panel
  - mumps
  - trichophyton
  - tetanus
  - candida

A positive test does not necessarily indicate active disease but does indicated past exposure.

TB Testing

- Must be interpreted in conjunction with:
  - Risk assessment
  - Radiography
  - Medical & diagnostic evaluations
Histoplasmosis Skin Test

- 80% positive in patients with ocular histoplasmosis
- Large number of normals will also test positive
- 18% risk of reactivation of macular lesions with testing
- NOT routinely recommended

Case WL 41 y.o. AAM

- VA: 20/25+ OD 20/200- OS PH: 20/70-, PAP: 20/70
- Pupils: direct & consensual, 2+ APD OS
- Extraocular motilities: FROM OU
- CVF: FTFC OD, OS
- IOP: 14 mmHg OD, 2 mmHg OS
- Anterior segment: 2+ cell & flare OS
- Posterior segment: WNL OD
CASE WL: Lab Results

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<thead>
<tr>
<th>Item</th>
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<th>Reference Range</th>
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<tbody>
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<td>GLUCOSE</td>
<td>107 H</td>
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</tr>
<tr>
<td>UREA NITROGEN</td>
<td>14</td>
<td>7.2 - 21</td>
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<tr>
<td>CREATININE</td>
<td>1.4 H</td>
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<td>SODIUM</td>
<td>139</td>
<td>136 - 145</td>
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<tr>
<td>POTASSIUM</td>
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Case WL: Lab Results

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<td>WBC</td>
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<td>RBC</td>
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<td>2.0 - 5.7</td>
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<td>HGB</td>
<td>15.8</td>
<td>13 - 17</td>
</tr>
<tr>
<td>HCT</td>
<td>46.8</td>
<td>40 - 51</td>
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<tr>
<td>MCV</td>
<td>90.3</td>
<td>82 - 99</td>
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<tr>
<td>MCH</td>
<td>30.5</td>
<td>27 - 34</td>
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<tr>
<td>MCHC</td>
<td>33.8</td>
<td>31 - 37</td>
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<tr>
<td>RDW</td>
<td>12.0</td>
<td>&lt;15.0</td>
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<tr>
<td>MPV</td>
<td>10.9</td>
<td>8.0 - 12.0</td>
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Case WL: Lab Results

<table>
<thead>
<tr>
<th>Item</th>
<th>Value 1-2</th>
<th>Reference Range</th>
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</thead>
<tbody>
<tr>
<td>CEA</td>
<td>1.1</td>
<td>0.5 - 5.0</td>
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<tr>
<td>PROSTATIC SPECIFIC ANTIGEN</td>
<td>1.38</td>
<td>0.1 - 4.0</td>
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<tr>
<td>ALKALINE PHOSPHATASE</td>
<td>144</td>
<td>50 - 136</td>
</tr>
<tr>
<td>ALT</td>
<td>60</td>
<td>10 - 65</td>
</tr>
<tr>
<td>AST</td>
<td>24</td>
<td>10 - 37</td>
</tr>
<tr>
<td>BILIRUBIN, TOTAL</td>
<td>0.50</td>
<td>0.2 - 1.0</td>
</tr>
<tr>
<td>QUANTIFERON-TB GOLD</td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>ACE</td>
<td>41</td>
<td>9 - 67</td>
</tr>
<tr>
<td>LYSOZYME, SERUM</td>
<td>8.5</td>
<td>4.0 - 10.3</td>
</tr>
<tr>
<td>LYMEO POLYVALENT</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>RPR</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
</tr>
</tbody>
</table>

Case WL: Diagnosis

- Choroidal tuberculoma with diffuse serous retinal detachment, papillitis, and anterior uveitis

Histocompatibility Locus Antigen
Histocompatibility Locus Antigen (HLA)
- Glycoproteins on cell surface
- 27+ antigens in man
- Determines donor/recipient compatibility with organ transplants
- Associated with disease susceptibility
- **BUT does NOT mandate disease development**

Which test do I order?
- Cost
- Reference range
- Sensitivity
- Specificity
- Predictive Value

Special Considerations
- **Elderly**
  - lack of age-related standards
  - multiplicity of diseases
  - polypharmacy

Laboratory Caveats
- Lab testing does not replace a careful history & physical examination.
- Abnormal values may bear repeating
- Normal values do not always rule out disease
- Multiple diseases may co-exist
- Understand test limitations/interpretations

DH 34 y.o. AA Male
- Progressive vision loss x 2 months
- PMHx: Bell’s palsy 3 years prior
  crack cocaine addiction
- POHx: unremarkable
- BVA: 20/400 OD      HM @ 3ft. OS
- Pupils: reactive with 1+ Left APD
- Slit Lamp:
  - Few KP OD, OS
  - 1+ cell OD, OS
**Case LH: Slit Lamp**

- ** Conj:** OD: 3+ diffuse injection/circumlimbal flush  
  OS: 1+ diffuse injection  
- ** K:** OD: microcystic edema  
  diffuse (60-100 med/lg) mutton fat KP (hazy view)  
  OS: numerous small KP  
- ** A/C:** OD: difficult view but + cell and flare  
  OS: 1+ cell and 1+ flare  
- ** Iris:** OD: + nodules  
  OS: no nodules  
- ** Focal areas of posterior synechiae OU**
Case LH 42 y.o. AA Male

- **DFE**
  - C/D: 0.4V/H OD & 0.5V/H OS
  - ON: NL OU
  - Macula: grossly NL OD, OS
  - Vitreous: vitreal puff balls inferiorly OU
  - Periphery: grossly NL OD
  + 7-10 choroidal granulomas OS
  + inferior venous sheathing OS

Case LH: Lab Results

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>Reference Range</th>
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</thead>
<tbody>
<tr>
<td>RHEUMATOID FACTOR</td>
<td>26.7 H IU/mL</td>
<td>0 - 15</td>
</tr>
<tr>
<td>ANA</td>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>RPR</td>
<td>NR</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>ACE</td>
<td>200 H U/L</td>
<td>9 - 67</td>
</tr>
</tbody>
</table>

Comment: >> RESULTS RECHECKED

LYSOZYME, SERUM       | 13.5 H mcg/mL | 4 - 10.3        |
WESTERGREN*           | 58 H mm/hr    | 0 - 15          |

Case LH

- Elevated ACE & Lysozyme with clinical findings point to sarcoid
  - Confirmed with CXR
- ESR non-specific for inflammation
- Why is RF positive?

Case PB 49 y.o. AAM

- Recrudescent anterior uveitis OS>OD x 2 yrs
- Normal lab work-up at onset
  - including ACE, lysozyme, FTA, RPR, HLA-B27
- PMHx: chronic sinusitis
- BVA: 20/25 OD, 20/50 OS
- Slit lamp:
  - Circumlimbal injection OD>OS
  - 1+ cell and 1+ flare OD, 3+ cell and 1+ flare OS
  - + fibrin on K endothelium OS

Case PB

- All initially ordered labs were repeated and in addition PPD, ANCA, and HIV testing ordered…...
### Case: The Kitchen Sink

- **85 year old white male**
- **PMH:** cardiac arrhythmia, prostate CA s/p prostatectomy
- **BVA:** OD: 20/40, OS: 20/400
- **+ Left APD**
- **Slit Lamp:** unremarkable OU
- **2+ NS with central vacuoles OD, OS**

### Kitchen Sink

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<thead>
<tr>
<th>Test</th>
<th>Result</th>
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<tbody>
<tr>
<td>Foam Fractions</td>
<td>Reactive</td>
<td>Nonreactive</td>
</tr>
<tr>
<td>HIV</td>
<td>Non-reactive</td>
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</tr>
<tr>
<td>c-ANCA</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>p-ANCA</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HLA B5</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HLA B27</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HLA B51</td>
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</tr>
<tr>
<td>MHTATP</td>
<td>Reactive</td>
<td>Nonreactive</td>
</tr>
<tr>
<td>RPR QUANTITATIVE ACE</td>
<td>Reactive 1:128</td>
<td>Nonreactive 8 - 52</td>
</tr>
<tr>
<td>LYSOZYME, SERUM</td>
<td>11.0 U/L</td>
<td>4.7-14.5</td>
</tr>
<tr>
<td>PROTHROMBIN TIME</td>
<td>Reactive</td>
<td>Nonreactive</td>
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<tr>
<td>INR</td>
<td>1.33</td>
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<td>Reactive 1:20</td>
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<td>TRIGLYCERIDE</td>
<td>46 mg/dl</td>
<td>0 - 149</td>
</tr>
<tr>
<td>UREA NITROGEN</td>
<td>33 mg/dl</td>
<td>7 - 21</td>
</tr>
<tr>
<td>SODIUM</td>
<td>146 mmol/L</td>
<td>136 - 145</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>5.1 mmol/L</td>
<td>3.5 - 5.1</td>
</tr>
<tr>
<td>CHLORIDE</td>
<td>104 mmol/L</td>
<td>98 - 107</td>
</tr>
<tr>
<td>CO2</td>
<td>28.8 mmol/L</td>
<td>21 - 32</td>
</tr>
<tr>
<td>GLUCOSE</td>
<td>108 mg/dl</td>
<td>70 - 99</td>
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<tr>
<td>CREATININE</td>
<td>1.6 mg/dl</td>
<td>0.8 - 1.3</td>
</tr>
<tr>
<td>UREA NIT/CREAT RATIO</td>
<td>20 RATIO</td>
<td>6 - 23</td>
</tr>
<tr>
<td>PROTEIN, TOTAL</td>
<td>9.6 g/dl</td>
<td>6.4 - 8.2</td>
</tr>
<tr>
<td>CALCIUM</td>
<td>9 mg/dl</td>
<td>8.5 - 10.1</td>
</tr>
<tr>
<td>CHOLESTEROL</td>
<td>225 mg/dl</td>
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Don’t Forget
You’re Responsible!!

Unexpected Results

✦ How well do you know your laboratory?
  – Specimen handling
  – Laboratory error
✦ Test limitations
✦ Repeat testing
✦ Caution should be exercised to prevent overreaction to miscellaneous, mild abnormalities without clinical correlate
  – Consider Case history & clinical findings
✦ Look for trends