Clinical Hematology Review

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Clinical Hematology Review Part 1: General Hematology; RBC Evaluation
(as presented at the 2012 Louisiana/Tennessee/Mississippi Joint State Meeting, Tunica, MS)

Clinical Hematology Review Part 2: Leukocyte Evaluation
(as presented at the 2012 Louisiana/Tennessee/Mississippi Joint State Meeting, Tunica, MS)

From: http://cls.umc.edu/COURSES/CLS322/index.html
Part I
General Hematology
RBC Evaluation
Computer Adaptive Testing
• **Recall** [Level 1]
  verbs: List, Name, Identify, Show, Define, Recognize, State, Count, Find, Label, Match, Record, Select, Sequence

• **Interpretative skills** [Level 2]
  verbs: Summarize, Explain, Interpret, Describe, Compare, Differentiate, Convert, Discuss, Estimate, Give examples, Locate, Predict, Contrast, Arrange, Evaluate

• **Problem solving** [Level 3]
  verbs: Solve, Calculate, Apply, Classify, Assess, Choose, Compute, Determine, Develop, Prepare, Utilize, Outline, Diagram, Characterize, Perform, Resolve
Computer Adaptive Testing

Software Selects Question Area/Topic
Begin with Moderate (Level 2) Question

Student Response
Computer Adaptive Testing

Correct response
Wrong response

Harder item (Go to Level 3)
Easier item (Go to Level 1)
Computer Adaptive Testing

Correct response → Harder item

Wrong response → Easier item
Computer Adaptive Testing

• Correct Responses produce a SHORTER, but HARDER examination.

• Incorrect Responses produce a LONGER, but EASIER test.

• It is predetermined by the exam writers how many questions, and at what level of difficulty, MUST be answered by the student to PASS the exam.
A long standing “bug” with the old Registry was as follows: If a student made multiple incorrect responses, the exam questions were adjusted to lower difficulty, but did not stop after a fixed number of questions within a subject area, such as Micro. Therefore a student might have a very long, easy exam, with most of the questions being in Micro, possibly only two or three in other subjects, (Chemistry, Blood Bank)…
Computer Adaptive Testing

…and then FAIL the exam!

It is unclear to me whether this has been fixed in the new BOC, as students are still reporting that they have “lots of questions in one area, and just a few in others”.
Computer Adaptive Testing

Advantages

• Test is adjusted to the ability level of the examinee.
• In theory, a “more fair” test?
• Test security is maximized:
  Variety of test items
  Less passing of item information among students
Computer Adaptive Testing

Advantages

• Random order of content
• Student MUST answer when presented
  (i.e.: can’t return at the conclusion of the exam to answer a question)
General Hematology
Composition of Blood

- 55% liquid fraction
- 45% *Formed Elements*
  - red cells *erythrocytes*
  - white cells *leukocytes*
  - platelets *thrombocytes*
Functions of Blood

- Gas transport
  - Oxygen from lungs to tissues
  - Carbon dioxide from tissues back to lungs
- Nutrient Distribution
- Collection of Metabolic Waste
  - urea, creatinine, uric acid, etc..
Functions of Blood

- Carries Hormones of the ductless glands 
  i.e. thyroid, parathyroid 
- Maintains Fluid Content of Tissues 
- Helps Regulate Body Temperature
Blood Volume

- The average adult body contains approximately 6 quarts of blood (5 liters). This volume obviously varies with the size and health of the individual.
Blood Plasma

- Liquid fraction of blood
- Clear, straw colored
- Largest constituent is *protein*
- Substances dissolved in the plasma are known as *solute*
Plasma Solutes

- Comprise about 10% of plasma volume
- Major: Protein
- Minor constituents
  - nutrients
    - glucose
    - lipids
    - amino acids
Plasma Solutes

(Minor Solute Constituents, continued)

- metabolic end products
  - uric acid
  - urea
  - lactic acid
  - creatinine

- gases
  - oxygen
  - carbon dioxide
Plasma Solutes

*(Minor Solute Constituents, continued)*

- hormones
- enzymes
- antibodies
The Bone Marrow

- Develops in the embryo by the hollowing out of the skeletal bones forming a central cavity
- In this cavity develops a primitive, undifferentiated cell known as a hemocytoblast, or stem cell
- ALL blood formed elements ultimately develop from this undifferentiated precursor
The Bone Marrow

- Blood cells develop in what is called red marrow. It comprises approximately 50% of the marrow cavity space. The remaining 50% of the space is occupied by fat and is known as yellow marrow. The ratio of red marrow to yellow marrow is an indirect representation of marrow activity, and is expressed as marrow cellularity.
The Bone Marrow

General Characteristics of cell development

- most immature cells are largest
- immature cells possess *nucleoli*
  - nucleoli are structures within a nucleus which are composed primarily of RNA and are involved in mitotic processes
- granules, if present are acquired later in maturation
- erythrocyte nuclei are lost late in development
The Bone Marrow

- Developing cells are held within normal marrow until they have developed sufficiently to function normally. Then they are released into the circulating blood for the remainder of their life span.
The Bone Marrow

- Marrow recedes during development of the individual, and in the adult occupies only the support skeleton and the proximal regions of the long bones
Blood Cell Development

- **Primitive Stem Cell**
  - Most Immature Cell (hemacytoblast)

  - **Erythrocyte Series**
    - Red Cell Development
  - **Leukocyte Series**
    - White Cell Development
  - **Thrombocyte Series**
    - Platelet Development
Erythrocytes

- The normal red cell is called an *erythrocyte*
- It exists as a biconcave disk approximately 7 microns in diameter
- It is non-nucleated
- Contains *hemoglobin*
- Major function-oxygen transport
Erythrocytes

- Normal range 4.2-5.5 million per mm$^3$ in adults.
- Biconcave shape.
- Diameter 7 microns.
- Cells for transport of $O_2$ and $CO_2$.
- Life span 120 days.
Leukocytes

- Normal range 4 - 11 thousand per mm$^3$ in adults.
- Five types.
- Size 8-20 microns.
- Involved in fighting infection, combatting allergic reactions, and immune responses.
Leukocyte Groups

Granulocytes

- *polymorphonuclear* (multi-lobed) nucleus
- rather large granules in cytoplasm of cell

**Neutrophil**

- granules are neutral in color, brownish or tan
- 65% of WBC in peripheral blood

**Basophil**

- granules take up basic dyes, stain dark blue or black

**Eosinophil**

- granules take up acidic dyes, stain red or pink
Neutrophil Segmented Cell
Eosinophil
Basophil
Leukocyte Groups

Agranulocytes

- single, round or horseshoe shaped nucleus
- no large granules

**Lymphocyte**

- 20-25% of WBC in peripheral blood
- rounded nucleus
- function in immune defense

**Monocyte**

- 3-8% of WBC
- phagocytic cell
Monocyte
Lymphocyte
Rubriblast
Prorubricyte
Rubricyte (late)
Metarubricyte
Diffusely Basophilic RBC
Reticulocyte
Myeloblast
Promyelocyte
Myelocyte
Metamyelocyte
Band Neutrophil
Segmented Neutrophil (PMN)
Thrombocytes

- Smallest cells in the blood.
- Normal range 130,000-400,000.
- Active role in coagulation and hemostasis.
Megakaryocyte

Kyoto Univ.
Megakaryocyte
Platelet Function

- Adhere to vessel *endothelium* on injury
- *Aggregate* or clump together with other platelets
- Clump of platelets forms a “platelet plug”, helping to stop blood flow
- Secrete soluble substances which facilitate hemostasis and coagulation
The Reticuloendothelial System

- Functional, rather than anatomic classification.
- Highly *phagocytic* cells which serve to remove foreign materials from the blood, lymph, and tissues.
- Macrophages (*phagocytes*) are found throughout the body.
The Reticuloendothelial System

- Macrophages are produced from the bone marrow stem cell or hemacytoblast.
- They are released from the bone marrow as monocytes, which circulate for a few days in peripheral blood, then exit the circulation and settle in tissues as mature fixed, tissue macrophages (histiocytes).
- Process of exiting the circulation is known as diapedesis.
RE System Associated Organs

- Primary Lymphatics
  also called *Central Lymphoid Organs*

  *thymus* gland
  - responsible for normal development of some of the lymphocytes
  - located in the neck
  - maximum development in childhood, atrophies with age

  *Bursa Fabricus* - found in birds with possible analogous tissue in man. Responsible for normal antibody production
RE System Associated Organs

- Secondary Lymphatics
  also called Peripheral Lymphoid Organs
  *
  lymph nodes - oval structures distributed throughout the body connected by lymphatic vessels which carry a fluid called lymph.
  *
  spleen
  *
  GALT - gut associated lymphoid tissue
  *
  tonsils
  *
  Peyer’s Patches
Lymph Nodes and Vessels

- Lymphatic Vessels
  - form a network throughout the body
  - structure resembles that of veins
  - valves prevent backflow of fluid
  - collect proteins and water which seeps out of blood vessels into tissues and returns it to the peripheral blood
  - possess *nodes* or glands along their course
Lymph Nodes and Vessels

- Lymph Nodes
  - enclosed in fibrous capsule
  - identified by their locations
    - *cervical* - neck
    - *submental/submaxillary* - mouth
    - *clavicular* - above and below clavicles
    - *axillary* - axilla
    - *cubital* - elbow
    - *inguinal* - groin
Lymph Nodes and Vessels

- The lymph nodes act as filters to remove foreign blood contaminants. Extremely important part of the body’s infection defense
- Contain many phagocytic cells and lymphocytes
- Immature lymphocytes produced in the bone marrow collect and mature in the tissues of the nodes
Circulation of Lymph

- Lymph fluid develops from plasma which has escaped the vasculature.
- *Afferent* lymphatic channels carry lymph fluid to nodes.
- *Efferent* channels carry lymph away from nodes.
- Channels empty into the *thoracic duct*, which directs the fluid into the *subclavian vein*.
Circulation of Lymph

- Lymphocytes can escape vasculature by *diapedesis* through the walls of the post-capillary venules.
- They travel through the tissues eventually finding their way into lymph channels by the same process.
- Circulation of the lymph returns the lymphocytes to the peripheral blood.
The Spleen

- Reticuloendothelial organ
- Primary functions - *lymphopoiesis*, *phagocytosis*
- Located in the left side of the abdomen just below the diaphragm and behind the fundus of the stomach
- Largest structure of the lymphoid system
The Tonsils

• one of the Gut Associated Lymphoid Tissues (GALT)

• Small round masses of lymphoid tissues that function in blood filtration, as does the spleen and other reticuloendothelial organs

• *palatine* - back of the throat
  
  *lingual* - root of the tongue

  *pharyngeal* - (adenoids) roof of the pharynx
Routine Hematology

**Anticoagulant of choice: EDTA**

- Complete Blood Counts (CBCs)
- Manual WBC Differentials
- Erythrocyte Sedimentation Rates (ESRs)
- Sickle Screens
- Reticulocyte Counts
Automated Counting

**Coulter Principle**

Electrical impedance: resistance or change in current when cell passes between two electrodes in NaCl solution.

**Optical Principle**

change in current when cell blocks light path
Automated Counting

Flow Cytometry

Uses lasers to measure both forward and side scatter.

Forward scatter measures size.

Side scatter measures granularity.

Fluorescence detectors
Sources of Error

• *Inadequate mixing of specimen.*
• Hemolyzed specimens.
• Lipemic specimens.
• Cold agglutinins.
• Clotted specimens.
• Platelet clumps or platelet satellitosis.
• Improperly diluted specimens.
Platelet agglutination

- Most frequent cause of false low plt counts
- Large, may be counted as WBCs – falsely elevated WBC as a result
Know Normal Ranges!

{However recent reports have indicated that normal/reference ranges are now provided with the examination questions}

<table>
<thead>
<tr>
<th>WBC</th>
<th>PLT</th>
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<tbody>
<tr>
<td>RBC</td>
<td>MPV</td>
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<tr>
<td>HGB</td>
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<td>MCV</td>
<td>MONOCYOTES</td>
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<td>MCH</td>
<td>EOSINOPHIILS</td>
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<tr>
<td>MCHC</td>
<td>BASOPHIILS</td>
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</table>
RBC Morphology
Elliptocytes

Significant proportions (> 10%) of elliptical cells may be due to hereditary elliptocytosis or megaloblastosis. In megaloblastosis, the cells are also macrocytic (macro-ovalocytes).
Doubly biconcave. Viewed from the top (above) they resemble a target due to increased depth of hemoglobin in cell center. Significant numbers of target cells occur in three situations: (1) hepatobiliary disease (increased cholesterol accumulating in cell membrane); (2) hemoglobinopathies C, D, and E; (3) thalassemia.
Tear Drop RBC

Most numerous in myelofibrosis with myeloid metaplasia (MMM). They may occur in small numbers in nuclear or cytoplasmic maturation defects. In MMM the spleen is responsible for producing this shape change. Following splenectomy, tear-drop cells disappear.
Crenated/Burr Cell

Burr cells are commonly seen in uremia, chronic renal disease, and pyruvate kinase deficiency. However, red cells mimicking burr cells are more often seen as drying artifacts of thick smears or with the use of aged blood.
Air Space Under Coverslip (artifact)
Rouleaux formation as an artifact is generally seen in the thick area of virtually all blood smears. However, true rouleaux formation is due to hyperproteïnemia (primarily fibrinogen and globulins) and is recognized in the thinly spread area of the smears, where normally the red cells barely touch one another. It is often associated with proteinaceous (blue staining) background.
Malarial Parasites in RBCs

Intraerythrocytic parasites (e.g., plasmodia) are beyond the scope of this discussion, but may often be confused with other intraerythrocytic inclusions.
Microcytosis and Hypochromia

Microcytes are RBCs with significant decrease in diameter. Normal erythrocytes tend to have a central pale area that is less than 1/3 of the cell diameter. Hypochromia is present when the pale area is larger than this.
Stomatocytes are erythrocytes with an elongated (mouth-like) area of central pallor. An occasional cell of this type might be seen as a non-specific finding in a variety of situations, such as regenerative anemias, liver disease, and lead poisoning. Stomatocytes can also be an artifact in a blood smear that is too thick.
Spherocytes

A prehemolytic form seen in hereditary spherocytosis or as an acquired change. Acquired microspherocytosis is seen most frequently in immunohemolytic anemias (autoimmune hemolytic anemia, ABO erythroblastosis fetalis, hemolytic transfusion reaction); in patients with severe burns; and with certain toxins (e.g., Cl. welchii exotoxin).
Schistocytes (Helmet Cells)

Portions of such cells have been lost by mechanical fragmentations. They are prehemolytic forms. Unlike microspherocytes, cells of this type are always due to extrinsic defects (e.g., endothelial disease).
Acanthocyte

RBCs with multiple, irregular, spinelike projections over their surface. Seen in abetalipoproteinemia and occasionally in severe liver disease.
Sickle Cells

Blood films from patients with homozygous hemoglobin S disease frequently have sickle cells, but their absence does not exclude that diagnosis. Individuals with S trait rarely have sickle cells on routine blood films. Double heterozygotes (e.g., hemoglobin SC) may or may not have sickle cells on blood film.
Schistocytes

Fragment of an erythrocyte. Schistocytes are typically irregularly shaped, jagged, and asymmetrical. True schistocytes do not have central pallor.
RBC
Inclusions
Howell-Jolly Body

nuclear remnant; spheroidal, between 0.5 and 1.5 micra in diameter. Rarely is there more than a single Howell-Jolly body per erythrocyte. Commonly seen after splenectomy.
Metarubricyte (not an inclusion, however MAY BE confused w/one)

Nucleated RBC containing pyknotic nucleus ready for extrusion.
Hgb C Crystals

Polygonal inclusions displacing the hemoglobin in Hgb CC. Crystals nearly fill the cell and distort its shape. Inclusions also occur in individuals doubly heterozygous for Hb C (e.g., Hb SC disease), are not seen in Hb C trait.
Hgb SC w/partial crystals & Target RBC
Basophilic Stippling

- Numerous, small purple inclusions in RBCs.
- Aggregates of ribosomal RNA.
- Most commonly seen in lead poisoning.
Pappenheimer Bodies

- Clusters of dark blue granules, irregular in size and shape.
- Composed of iron and ribosomal RNA.
- Seen in sideroblastic and hemolytic anemias.
Classifications of Anemias
Classifications of Anemias

**Microcytic, Hypochromic**
- Iron deficiency
- Sideroblastic
- Chronic disease, Inflammation
- Lead poisoning
- Thalassemia trait
Microcytic, Hypochromic

- Many RBCs smaller than nucleus of normal lymphocytes, increased central pallor.
- Iron deficiency, thalassemias, anemia of chronic disease.
What disorder could this morphology indicate?

A. Iron deficiency anemia  
B. Folate deficiency  
C. Acute leukemia  
D. Infectious mononucleosis
What disorder could this morphology indicate?

A. Iron deficiency anemia
Classifications of Anemias

**Normochromic**
- Hereditary Spherocytosis
- Hereditary Elliptocytosis
- PNH
- G6PD deficiency
- Aplastic anemia
- Acute blood loss
Classifications of Anemias

**Macrocytic**
- Vitamin B12 deficiency
- Folate deficiency
- Liver disease
Macrocytic RBCs

- Most RBCs larger than nucleus of normal lymphocytes, increased MCV.
- Folate or Vitamin B12 deficiencies, alcoholism, and liver disease.
What disorder could the WBC morphology indicate?

A. Infectious mononucleosis
B. AML
C. Pernicious Anemia
D. Bacterial infection
E. Multiple myeloma
What disorder could the WBC morphology indicate?

C. Pernicious Anemia
What is the best description of the WBC?

A. Variant lymphocyte
B. Pyknotic nucleus
C. Hypersegmented
D. Toxic granulation
E. Vacuolization
What is the best description of the WBC?

C. Hypersegmented
Reticulocytes

- Immature RBCs.
- Contain residual ribosomal RNA.
- Reticulum stains blue using a supravital stain (new methylene blue).
- Counted and expressed as % of total red cells.
Reticulocyte Count

Uses supravital stain which stains cells in the living state.

Retic % = \( \frac{\text{# retics per 1000 RBCs}}{10} \)

Corrected retic= \( \frac{\% \text{ retics x pt. HCT}}{45} \)
Hemoglobinopathies

*Beta Chain Substitutions*

Hgb S: Valine for glutamic acid
(6th position, beta chain)

Hgb C: Lysine for glutamic acid
(6th position, beta chain)
Hemoglobinopathies

<table>
<thead>
<tr>
<th>Alkaline Electrophoresis</th>
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</table>
What hemoglobin abnormality is suggested by this peripheral blood smear?

A. CC
B. AS
C. SS
D. AC
E. SC
What hemoglobin abnormality is suggested by this peripheral blood smear?

C. SS
If this cell morphology was observed, the next step would be to perform:

A. Hemoglobin electrophoresis.
B. A bone marrow iron stain.
C. Kleihauer-Betke stain.
D. An erythrocyte sedimentation rate.
If this cell morphology was observed, the next step would be to perform:

A. Hemoglobin electrophoresis.
The Smiths have two children. Mr. Smith has sickle cell trait and Mrs. Smith hemoglobin C trait. Their daughter is healthy, but a number of target cells were seen on her peripheral smear. Their son has a hemolytic anemia requiring blood transfusions. Which of the following genotypes are likely for the two children?

A. AA and SC
B. AC and SC
C. AS and SC
D. AS and AC
E. SS and AC
### Possible Offspring

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>C</th>
<th>Mrs. Smith</th>
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<tbody>
<tr>
<td>S</td>
<td>AS</td>
<td>SC</td>
<td>Double heterozygote</td>
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<tr>
<td>C</td>
<td>AC</td>
<td>CC</td>
<td>Hgb C Disease</td>
</tr>
<tr>
<td>Mr. Smith</td>
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</tbody>
</table>

- **AS**: Asymptomatic carrier
- **SC**: Double heterozygote
- **AC**: Asymptomatic carrier
- **CC**: Hgb C Disease
The Smiths have two children. Mr. Smith has sickle cell trait and Mrs. Smith hemoglobin C trait. Their daughter is healthy, but a number of target cells were seen on her peripheral smear. Their son has a hemolytic anemia requiring blood transfusions. Which of the following genotypes are likely for the two children?

B. AC and SC
Cellulose Acetate Electrophoresis

Which lane represents the symptomatic son?

{Will not separate A&C, S&D}
Citrate Agar Electrophoresis

- pH 6.0-6.2
- Will separate A & C
- Will separate S & D
Which of the bands here is representative of the symptomatic son?
Which of the bands here is representative of the symptomatic son?

Right! SC
Which of the bands here is representative of MR. Smith (Sickle Trait)?
Which of the bands here is representative of MR. Smith (Sickle Trait)?

Right! AS
Citrate Agar Electrophoresis – additional case
Which lane represents the blood film shown?
Citrate Agar Electrophoresis – additional case
Which lane represents the blood film shown?

Hgb SC Double Heterozygote
An African American woman presents with a history of intermenstrual bleeding. Her gynecologist ordered a blood count which showed a Hb 20.0 g/dl, normal white cell count and platelet count and normal morphology.

Hb electrophoresis on cellulose acetate at pH 8.4
1. Normal newborn with Hb Barts
2. Hb C disease
3. Hb SC
4. The patient.
5. Hb S trait in newborn
Diagnosis: Hb SN, double heterozygote for Hb S (the solubility test was positive) and Hb N Baltimore.

Comment: There are equal amounts of Hb S and the fast migrating Hb N (about the same speed as Hb Barts) Hb N has a beta chain abnormality. Hb N acts like normal Hb A. therefore this combination is similar to Hb S trait.

Why did the patient have such a high hemoglobin?
Why did the patient have such a high hemoglobin?

The gynecologists removed a large uterine tumor (lyomyosarcoma).

Within a few months the patient's hemoglobin returned to normal levels.

Final diagnosis. Erythropoietin-producing lyomyosarcoma in a patient doubly heterozygous for Hb S and Hb N.
What disorder could this morphology indicate?

A. Iron deficiency anemia
B. Hereditary elliptocytosis
C. DIC
D. Infectious mononucleosis
What disorder could this morphology indicate?

C. DIC
What further lab tests are suggested by this morphology?

A. Serum iron and ferritin
B. Genetics carrier screening
C. Coag panel
D. EBV titers
E. Hgb electrophoresis
What further lab tests are suggested by this morphology?

C. Coag panel

(PT, PTT, fibrinogen, FDP, D-Dimer)
Schistocytes

Can cause spurious increases in platelet counts
Case 1

- 68 y.o. woman from Minnesota seen in ER for fatigue, DOE, and episodic dark urine. Dark urine noted following extended periods of time in the cold. No medications
- PE: T nl, HR 90, BP 110/75
- Hgb 7.1, retic count 18%. Normal indices, normal remainder of CBC
- Lab notes sample appears agglutinated, and clumping of RBCs on smear.
Case 1

• 68 y.o. woman from Minnesota seen in ER for fatigue, DOE, and episodic dark urine. Dark urine noted following extended periods of time in the cold. No medications

• PE: T nl, HR 90, BP 110/75

• Hgb 7.1, retic count 18%. Normal indices, normal remainder of CBC

• Lab notes sample appears agglutinated, and clumping of RBCs on smear.
Case 1

Which of the following is the most important study for establishing the diagnosis in this patient?

– A. Osmotic fragility
– B. Cold Agglutinin screen
– C. Serum complement determination
– D. Sucrose hemolysis test
– E. G6PD determination
Case 1

A - Osmotic fragility - **No**, since this is for hereditary spherocytosis.

Other clues for Hereditary Spherocytosis

- Recurrent jaundice
- Early pigment gallstones, cholecystectomy
- Autosomal dominant, so family history
- Splenomegaly
Case 1

B - Cold agglutinin Screen. Yes

Clues for cold agglutinin disease:

- Recurrent hemolysis in the cold
- RBC clumping on smear
- DAT (+) for C3/complement, (-) for IgG
- May follow infection with mycoplasma or mononucleosis
- IgM mediated
- No benefit from steroids or splenectomy
- Keep warm
C. Sucrose Hemolysis test. **No.** This is for PNH

Clues for Paroxysmal Nocturnal Hemoglobinuria
- Dark/red urine in early AM
- May have clots and/or pancytopenia
- May follow chemotherapy/aplastic anemia
- Tests:
  - sucrose hemolysis test
  - Acidified serum hemolysis test (Ham’s test)
  - Flow cytometry for CD55/CD59
  - Urine hemosiderin positive
Case 1

E. G6PD determination  No.

Clues for G6PD deficiency

– African-American or Mediterranean
– X-linked
– Hemolysis follows infection or drugs
– Susceptible to aplastic crisis after Chronic parvovirus infection
– Drugs - sulfa/dapsone/antimalarials
– Heinz bodies (need special stain)
– Bite cells

Blister cells
More Hemolytic Anemia Clues

• Warm AIHA
  – Spherocytes
  – Positive DAT, IgG positive, C3 positive
  – Associated with CLL, non-Hodgkin’s Lymphoma, SLE
  – Treat with steroids first, splenectomy second.

• Drug-related AIHA
  – Spherocytes.
  – Positive DAT, usually for C3 only.
Case 2

- A 22 year old man was brought from work to emergency department for abdominal pain and fever.
- Two recent episodes of red urine
- Previously treated for syphilis
- Works as a butcher’s assistant, unloading refrigerated meat trucks
- PEx - chronically ill. NI Temp and BP
Case 2

– A 22 year old man was brought from work to emergency department for abdominal pain and fever.
– Two recent episodes of red urine
– Previously treated for syphilis
– Works as a butcher’s assistant, unloading refrigerated meat trucks
– PEx - chronically ill. Normal Temp and BP
Case 2

Laboratory studies

- Hgb 4.0
- Plts normal
- WBC normal. Normal diff except for NRBCs
- Rapid Plasma reagin test - positive
- Urinalysis: Strongly positive for hemoglobin.
  No intact erythrocytes
Case 2

Which of the following is the most appropriate diagnostic study for this patient’s hemolytic anemia?

- A. Donath-Landsteiner test
- B. Sickle Cell Preparation
- C. Urine Hemosiderin preparation
- D. Heinz body preparation
Case 2

B. Sickle Cell preparation - No
   - Urine shouldn’t be red, may have microscopic hematuria, not hemoglobinuria
   - No association with syphilis
   - Peripheral smear should show sickled cells, especially if patient is acutely ill
Case 2

C. urine hemosiderin determination - No

– We know that there is hemoglobin in the urine.
– Urine hemosiderin would be useful if there is the suspicion of chronic intravascular hemolysis, as in PNH, valve hemolysis, Abdominal Aortic Aneurysm/aortic dissection hemolysis.
Case 2

D. Heinz body preparation - No

- Would be useful if there is suspicion of oxidative stress, usually from drugs.
- “bite cell” hemolytic anemia
- Usually G6PD deficiency, but if oxidative stress is bad enough, anyone can get Heinz body hemolytic anemia.
- African Americans with G6PD deficiency can have falsely normal G6PD levels immediately after hemolysis
Case 2

A. Donath Landsteiner test - correct
   - Confirm Paroxysmal Cold Hemoglobinuria (PCH)
   - Special test for DL antibody detection.
   - Episodic cold-induced intravascular hemolysis
   - DAT positive only for Complement
   - May be seen in pediatrics, also classically with syphilis, most are idiopathic
   - IgG antibody binds only in the cold, but fixes complement. No spherocytes
Case 3

• 22 y.o. man seen in the ER for red urine and fatigue. 3 days ago, started on TMP-SMZ* for UTI.

• Hbg 6.5 retic 18%

• Blood smear - polychromatophilia, blister cells

• G6PD - low normal

* Trimethoprim/Sulfamethoxazole, i.e. Bactrim, Septra
In order to confirm the diagnosis, which of the following should be done?

- A. repeat G6PD determination in 1 month
- B. perform osmotic fragility test
- C. perform sucrose hemolysis test
- D. perform bone marrow aspirate
Case 3

In order to confirm the diagnosis, which of the following should be done?

- A. repeat G6PD determination in 1 month
Case 3

- Two normal forms of enzyme. Most prevalent type is B. 20% of healthy Africans have type A.
- Deficiency of the enzyme is X-linked.
- In Africans, mutant allele is A-, which is unstable and loses activity as the red cell ages.
- Mediterranean variant has baseline low activity
- Low G6PD activity results in low levels of NADPH and reduced glutathione, which are required to protect hemoglobin from oxidative damage.
Case 3

– Typically, hemolysis can be triggered by drugs or infections.

– Anemia is maximal 7-10 days after exposure. In individuals with A-, production of reticulocytes begins to compensate for the anemia, despite continuation of the drug.

– Immediately after a hemolytic episode, G6PD levels in individuals with A- may be normal, since the mature cells have been lysed, and only younger cells with normal G6PD levels, are present. Need to repeat test in 1 month.
Case 3

• G6PD deficiency is associated with protection against malaria, notably in Africa where one form of G6PD deficiency (G6PD A-) is widespread.

• Protection is similarly seen in erythrocytes containing Hgb S, either AS or SS.

• These protections are particularly relevant in populations where, as one author noted, because “…virtually all young children experience episodes of malaria, such protective hemoglobinopathies and erythrocyte polymorphisms offer a tremendous survival benefit when they prevent progression of uncomplicated malaria.”
Case 3 - blister cells