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Notes 

Dear UAB Pathology Residents:

The Division of Laboratory Medicine would like to welcome you to your rotations in Clinical Pathology. During the next few months to years you will be rotating through Microbiology, Immunology, Transfusion Medicine, Coagulation, Chemistry, Molecular Diagnostics and Cytogenetics, Flow Cytometry, and Hematology. We expect that you will enjoy all of these rotations, as well as learn from them.

These resident survival guides are designed to help you answer the more common questions that may come up during your rotations and while on call. While we have tried to include all the pertinent information, there will clearly be other information that you wish were included. Please note this information down on the blank pages provided in the back of this manual.

Towards the end of this residency year, I will be asking you for your input to improve these manuals for next year. I will also be asking for feedback on your rotations and how we may improve them every year.

Please do not hesitate to contact me with any questions or suggestions.

Lance A. Williams, III., MD
Assistant Program Director for Clinical Pathology

Staff (Pathology & Hospital)		Email
ESTIS, Anita	4-6246	anitaestis@uabmc.edu
HEATON, Gina	5-8160	gheaton@uabmc.edu
HENDON, Diana, RN - (BB)	5-2513	dmhendon@uabmc.edu
INFORMATION SERVICES	4-6610	PathIS@uabmc.edu
LYLES, Cheryl	5-3286	clyles@uabmc.edu
MAIN LINE	4-4303	N/A
MARTIN, Debbie	5-8161	debramartin@uabmc.edu
PEEPLES, Ova	4-0578	opeeples@uabmc.edu
PHILLIPS, Brenda	12-4379	brenda.phillips@va.gov
SAMPLES, Jackie	4-4411	jbsamples@uabmc.edu
WHITE, Rea	4-7774	rpwhite@uabmc.edu

Departments	Phone	Location
Hospital Paging	205-934-3411	
UAB Hospital	205-934-4011	
When calling from within	4-XXXX	
Children's Hospital	205-939-9100	
When calling from within	9-XXXX	
Callahan Eye Foundation	205-325-8100	
The Kirklin Clinic	205-801-8000	
When calling from within	1-XXXX	
Laboratory Medicine	205-934-6421	West Pavilion P230
Lab Med Fax number	Fax 205-975-4468	
ARC-Ref Lab in Birmingham ARC in Charlotte for HLA matched platelets	205-994-7265 704-347-8205	
Blood Center of Wisconsin	1-800-245-3117	

Hospital Laboratories	Phone Numbers	Location
Blood Bank	4-6390 (Fax 5-9260)	SW W215
Blood Bank - Satellite Blood Bank - Highlands	4-8965; 6-7979 930-6771	7650A 3725
Chemistry Special Chemistry / Electrophoresis	4-6830 4-2854	SW S266
Coagulation	4-5385	SW S288
Diagnostic Molecular Laboratory	4-0452	SW S228
HLA Lab	4-4714	RWUH M250
Hematology	4-5625	SW S288
Bone Marrow Lab	4-7869	SW S281
Flow Cytometry	4-5615	SW W294
Immunology	4-4691	SW S234
Microbiology	4-4833	SW S218
Send-outs	4-6440	SW S299
NP Operating Room	5-5111	NP-5

UAB DEPARTMENT OF PATHOLOGY

Please visit the link below to see all weekly conferences held within the Department of Pathology.

http://www.uab.edu/medicine/pathology/images/forms-pdfs/ConferenceSeminars/05-23-2016calendar.pdf

***Note: For conferences that are mandatory under a specific rotation, please look at the specific Survival Guide that pertains to the rotation that you are on.**

CLINICAL PATHOLOGY Conferences

Division-wide Conferences - (required attendance for residents on CP rotations).

Laboratory Medicine Noon Report
Monday at 12 Noon (WP P230C)

Laboratory Medicine Seminar
Tuesday at 10:45 AM (WPCC-D, unless otherwise noted)

CHIEF RESIDENTS:

Dr. Alex Feldman - Chief Resident, Anatomic Pathology
Pager # 2039 - HSB 190
Phone # 6-2360
Phone # for Receptionist 4-4303
E-Mail: afeldman@uabmc.edu

Dr. Joseph Drwiega - Chief Resident, Clinical Pathology
Pager # 2035 - RWUH M294
Phone # 5-8171
Phone # for Receptionist 4-4303
E-Mail: jdrwiega@uabmc.edu

Rotation-Specific Conferences

Blood Bank

****Blood Bank Morning Call Report**

M, Tu, Th, F @ 8:30 AM; Wed @ 8:15 AM WP P230C

Blood Bank Didactic lectures

Monday at 12 Noon (July and August only - **Mandatory for PGY-1**), and Thursday @ 11:00 AM

Transfusion Service Quality Meeting

Quarterly

Blood Utilization Committee Meeting

4:00-5:00 PM on first Tuesday of every-other month

***Attendance strongly encouraged for all first years and the on-call resident. Upper level residents on CP rotations strongly encouraged to attend on Wednesdays.*

Microbiology/Immunology

Infectious Disease Conferences
Thursday: 8 AM (Case Conference)
Noon (ID Grand Rounds)

Clinical Microbiology Lab Meeting
Monday at 2 PM (WP P230C)

Clinical Immunology Lab Meeting
Wednesday at 2 PM (WP P230C)

End-of-Rotation Talk
Usually on Monday at noon during final month of rotation

Chemistry

Chemistry Electrophoresis Sign-out
Daily at ~1:30 PM (Chemistry Lab)

Clinical Chemistry Lab Meeting
Wednesday at 9 AM WP P230C

End-of-Rotation Talk
Usually on Monday at noon during final month of rotation

Molecular/Cytogenetics

Molecular pathology lies under both AP and CP. Please make your effort to attend AP conferences, such as APSS on Wednesday noon.

Molecular weekly QC/QA Meeting – Thursday at 10 AM
(WPCC-C)

Molecular Diagnostics Sign Out Session – Daily at 3 PM (time may be subjected to change)
(Molecular Diagnostic Laboratory)

Molecular Test Development Meeting – Monday at 11 AM
(WPCC-C)

Joint Molecular Genetic Pathology Conference –
4th Tuesday 4-5 PM, every other month (WCCC-D)

Molecular Tumor Board – Last Thursday of the month at 7:15 am (Rad Onc Conf Rm 2245)

Hematopathology

Hematology/Oncology/Pathology Case Presentation Conference
Monday at 8 AM (WPCC-Board Room)

Hematopathology Sign-out Session
Daily at 9 AM and 4 PM
(Bone Marrow Laboratory)

Hematopathology Tumor Board
Friday at 2:00 – 3:30 PM CCC Board Room

Lab Medicine Seminar

Seminar Description

This seminar series consists of two types of presentations – a Journal Club presentation and a LM Seminar format.

LM SEMINAR:

The LM Seminar format focuses on a specific question relevant to Lab Medicine. The title of the seminar should be a concise statement of the specific question to be answered. The seminar should present several key papers from the primary literature that will allow an evidence-based answer to the question posed. As much as possible, the question should be phrased in a way that the answer can be "yes or no", with appropriate qualifications as needed.

The essence of LM Seminar is **"what patient population, data, and statistical techniques did the authors use to answer the question at hand and how does that knowledge contribute to the larger question you are trying to answer?"**

The presentation should have a short focused introduction, followed by presentation of several papers with data that allow a data driven answer to the question. Although not every data figure in each paper needs to be presented, the details necessary to interpret the data presented must be shown.

At the end of the presentation, any relevant financial aspects necessary for a definitive answer to the questions should be presented. For questions involving a specific test, especially whether to bring a test in-house, the current usage data and costs should be obtained from the hospital lab administration and presented explicitly prior to your answer

to the question. The costs should include the price charged by a reference lab, the CMS reimbursement amount, and the reagent and expected labor cost for in-house testing. You need to request this information several days prior to your presentation. You can begin this request with the supervisor of the relevant hospital lab section, who may help you find further information with the lab administration.

The final segment of the presentation should be a clear answer to the question posed in the title of the seminar, along with your rationale for that answer. Also, you should acknowledge the help of the primary faculty mentor that helped you select the topic, papers, and review of the presentation.

For first and second year residents, it is required that the resident meet with Dr. Bucy **at least one and a half to two weeks prior** to the scheduled presentation. The Lab Medicine administrative assistants will contact the residents to schedule this meeting.

In addition to question/topic selection, initial faculty instruction is provided in critical thinking, data collection and evaluation (including use of common statistical test methods), presentation and communication skills. For subsequent seminars, the resident may seek the mentorship of any LM Faculty member, but it is still required to seek guidance beginning at least one week prior to the seminar presentation.

Objectives

In preparation for their responsibility as a practicing pathologist with an integral role in laboratory and hospital management:

- Residents will learn to identify and think critically about a current laboratory issue.
- Residents will gain graduated experience in systemic analysis of laboratory methods and problems, data analysis, statistical analysis, and presentation style / effectiveness.
- Residents will learn to effectively communicate problem description, analysis, and conclusions.

Faculty Instructors

R. Pat Bucy, MD, PhD - Seminar Instructor;
Laboratory Medicine Faculty – Preceptors

Schedule

The LM Seminar and Journal Club series will begin in August and be conducted weekly through the following June. Three instructional seminars will be conducted by Dr. Bucy at the beginning of the first semester. Presentations will be assigned during the resident's core clinical pathology (CP) rotation or CP elective.*

Evaluation

All seminars will be evaluated in writing by faculty and residents for the following: clarity of presentation, knowledge of subject, presentation style/organization, slide quality, introduction, data analysis, information integration and conclusions, and response to questions. Immediately following the presentation, the residents and Dr. Bucy will conduct a short review and evaluation of the presentation with the presenter.

Journal Club:

Journal Clubs will alternate with LM seminar presentations. Residents will select a current paper to present for discussion by all LM faculty and residents. The Journal Club presentation focuses on a single substantial paper from the current literature and a detailed presentation of the data in that paper. The resident presentation should also include references to other work in the field to give an appropriate context for the selected primary paper.

The chosen paper must be a substantial publication (more than 2 or 3 data Figures or Tables and published in a high quality journal) able to support a full hour in presentation and discussion. Papers presenting a meta-analysis of other papers can be discussed in the introduction, but should not be used as the primary paper for the presentation. Residents are also required to seek guidance for the selection of papers, as for the LM Seminar format, from Dr. Bucy for 1st year residents, and any LM Faculty member for subsequent residency years. This contact will be arranged **at least 1 week prior** by the Lab Medicine administrative assistants.

Attendance for Journal Club and LM seminars

Attendance is **required** at both Journal Club and LM seminars for all residents who are current on LM rotations. Failure to maintain an 80% attendance record based on the sign-in sheet will result in a report being filed with the residency program director, which will be reflected in your annual evaluations. You can have excused absences if you are out sick, on vacation, or away on official leave (attending a scientific meeting, etc.) that do not count in the total. You should notify the chief resident of such excused absences to communicate to LM office assistant who maintains the attendance records. You are welcome to attend these conferences when you are on an AP service and that attendance will count to increase your attendance record.

Weekly Clinical Pathology Rounds and Review

This weekly meeting at noon on Monday is attended by Laboratory Medicine faculty and all residents on Clinical Pathology rotations. At the beginning of the year, noon report consists of lectures by attending physicians to teach new residents about potential emergent or on-call issues that frequently arise. The remainder of the year consists of a mix of didactic lectures, end of rotation talks, and board review lectures.

End of Rotation Talk

Most of the CP rotations will require an end of rotation talk. These presentations are unrelated to the Laboratory Medicine Seminar lectures and have a different objective.

The primary goal of an end of rotation talk is educational and didactic in nature. The format for the talk is flexible and may vary among the rotations. In general, the talk should be 30 minutes or more in length and review a topic(s) from the resident's current CP section in greater depth. The resident

may present the material in the format they choose.
Potential suggestions include:

1) Unknown case(s) report
2) Didactic on a particular topic
3) Summation of high points of a pertinent chapter in Henry
4) Presentation of a pertinent journal article of interest

For further guidance, please discuss with the faculty director of your CP rotation.

CP Pathology Resident On-Call Sign-out

Before a resident rotating on CP leaves for the day, he/she must verbally notify the on-call resident of **any** pending issues. **This discussion must take place, even if it is only to say that there are no pending issues.** The handoff should be documented in an email to the on-call resident. The on-call attending and the CP chief resident should be copied on the email. The subject of the email should be "CP signout" so the emails can be sorted quickly. This handoff must include the opportunity to ask and answer questions. Issues to be discussed include but are not limited to:

- Ongoing apheresis procedures or potential apheresis procedures which are expected to take place after hours.
- If an apheresis consult is received before 7 pm, but will not be initiated until after 7 pm, the day resident is expected to begin and complete as much of the consult and preparation as possible. Any preparation not completed prior to 7 pm should be verbally signed off to the call resident.
- Apheresis consults received during on-call hours should be initiated and completed dependent upon the time the consult was received.

- Anticipated critical shortage (imminent triage status) of any blood product.
- Any patient requiring cross-matched platelets (i.e., CCI pending, expected arrival of cross-matched platelets, okay to give stock platelets in the meantime).
- Any issue regarding the administration of recombinant factor VIIa, PCC/KCentra, or any of the other coagulation factors. These orders, whether the product was administered already or not, usually print out again at around 2 am and result in a call to the on-call resident for approval. To prevent unnecessary phone calls in the middle of the night, the on-call resident should check with the clinical team and the blood bank early in the evening to ensure that the orders are clearly communicated to the night shift staff.
- Any issue regarding patients with clotting disorders requiring replacement, even if the patient is not anticipated to require a dose overnight. Please convey the patient's weight, the calculated dose, and the discussed dosing schedule. Please also convey to the on-call resident the type of replacement factor to be issued and the status of in-house stock for that factor.
- Any potential transfusion reaction(s) which occurred during the day that is/are still being investigated.
- Any issue where an exception is being made to an established lab protocol (i.e. washing red blood cells for non-standard reasons, administration of coagulation factors without a confirmed diagnosis of a clotting disorder, etc.)

Hematology Lab (UAB) / Hematopathology

Evening and Weekend Call Issues

Following is the list of the potential evening and weekend resident calls from hematology / BM lab (UAB).

On all of these calls, attending/on call pathologist should be consulted first, and if needed, contact Dr. Reddy or designated pathologist / hematopathology fellows.

Type of Call	Standard Operating Procedures
1. New patients with blasts > 5% or organisms in CSF	Main laboratory / Hem lab technologists will page and notify Lab Medicine resident on call on all "new" patients with blasts > 5%, resident should contact the clinical team (ER, main hospital, TKC and Highlands etc.) and inform / discuss the increase of blasts on smear review. Residents do not have to come to the lab for smear review, however, should inform and document discussion with the clinical team. Similar process applies to CSF organisms. Only exception is where the confirmation/ or diagnosis is needed for immediate treatment. Resident may escalate the call to faculty backup and or page Drs. Reddy (pager # 0331), Peker or Bucy.

2. Evening and weekend bone marrows	***Not offered*.** Rare exceptions are for the patients requiring immediate treatment. Page Dr. Reddy or designated pathologist for instructions/approval.
3. Specimen for flow cytometry analysis and cytogenetics	Holding media (Hanks/RMPI-1640) media is available in bone marrow lab (281B – Spain). Blood or bone marrow sample (1-2cc) are preserved in media in the refrigerator (4^{o}C). Check with Hempath fellows or Dr. Reddy.
4. Intracellular organisms	Residents are paged by hem-lab for confirmation / clinical correlation. **Note**: Slides are usually reviewed during day-time, however, if immediate confirmation is needed, the resident will review and confirm the findings with backup by senior resident, on call pathologist or Dr. Reddy. Resident must notify house staff / primary physician for appropriate additional tests (Gram's stain or cultures etc.).*Notification of the primary physician is documented in Hematology Section Log book and in Lab Med Consult form.*

Type of Call	Standard Operating Procedures
5. Atypical cells, blasts/tumor cells in body fluids or in peripheral blood.	Residents are paged by hem-lab for confirmation / clinical correlation. **Note**: In most cases, telephonic notification of the house staff / primary physician is sufficient. *In rare cases,* if the situation warrants, the resident will review and confirm the findings along with backup by senior resident, pathologist on call and or Dr. Reddy. *Notification of the primary physician is documented in Hematology section Log book and in Lab Med Consult form*
6. Crystal identification (urine or body fluid)	Residents are paged by hem-lab for confirmation. Note: Clinical correlation is needed in most cases and actual review of the smear is done during day time "slide review" session by hematology residents and Dr. Reddy.

Telephone and Pager Numbers

Labs	Telephone
Bone marrow lab (SW S281)	934-7869 7:00 am – 4:00 pm (M-F)
Routine Hematology Lab (UAB)	934-5625 - 24 hrs
Flow Cytometry Lab	934-5615 8:00 am – 4:30 pm (M-F)
Hematopathology Fellows	UAB Paging operator
Dr. Reddy	UAB pager # 0331, 24 hrs

Microbiology

1) Consults

Microbiology residents are occasionally consulted by the VA microbiology laboratory. As for consults from the UAB microbiology laboratory, please respond promptly and consult your attending as necessary. Maintain a record of the consult on the routine resident consult sheet.

How to handle a page alerting you that Cerner is down and is expected to be down for a prolonged time:

1) Ask if there is a need for resident intervention:
    A. Triaging phone calls from clinical staff, so the technologists can focus on operational details
    B. Help with paperwork
2) Notify the attending on call.
3) If the Cerner downtime persists into the normal work day, then notify the Chief Resident. The Chief Resident will notify all residents of the problem with Cerner. After notification, each resident should contact the laboratories they are rotating through and offer assistance. The Chief Resident will also be responsible for notifying all of the laboratory section heads of the Cerner downtime. This can be done via e-mail, page, or phone.

Dress Code:

Use good judgment at all times regarding your personal appearance. You are expected to dress appropriately, to be neat, to wear clean clothing, and to be careful with your personal hygiene. While in the laboratories you should wear a white coat over your street clothes.

For safety reasons, we have a very strict dress code for shoes worn in the labs. Part of that policy requires that toes be covered.

The policy applies across the board to everyone in the lab area: employees, visitors, etc., and is dictated by NCCLS nationwide for all laboratories.

Clinical Chemistry Survival Guide

2017-2018

FREQUENTLY USED PHONE NUMBERS

Department Fax: 975-4468
Central Page: 934-3411
Sendouts: 934-6440

CHEMISTRY PERSONNEL AND TELEPHONE NUMBERS

UAB	**934-6421**
UAB Hospital Laboratory	***934-6520***
Faculty:	
Professor and Section Head of Clinical Chemistry Robert W. Hardy, PhD Admin Contact: 934-0589	934-9925
Professor, Division of Laboratory Medicine John Smith, MD, PhD Admin Contact: 934-0589	934-7783
Professor, Division of Laboratory Medicine Andrew Robinson, PhD Admin Contact:	934-0589
Supervisors:	
Hematology Belinda Hyche	975-4663
Chemistry Lab Specimen Receiving Lab	934-6830
Diane Hendricks Administrative Director of Hospital Labs	996-1900
Sherry Polhill Administrative Manager of Laboratory Medicine	975-7380
Donna Scott Quality Manager	996-9698 934-6520 or 934-4872

VA 933-8101

Faculty
Leona Council 934-0062 or VA 933-810 Ext. 4739

Laboratory
Ina Walker 933-8101

Children's Hospital 939-9100
Faculty
Dr. David Kelly 939-9638
939-9636

Laboratory
Beth Thomas 939-9611

The Kirklin Clinic 801-8000

Faculty
C. Bruce Alexander, MD 934-2492 or 934-4303

Laboratory
Rachel Matta 801-8720

Administrative Manager
Paula Evans 801-6402 or 801-6401

Satellite Laboratories
Manager: Paula Evans 975-8108
Supervisor: Lela Beard

Anesthesiology/CICU Labs
Rhonda Boyd 934-5320

Critical Care Transport Lab
Under Point of Care Testing see below

ER Laboratory
James Knox 934-5320

RNICU Laboratory
Susan Roberts 975-8573 or 934-7310

Office of Bedside Testing
Faculty
C. Andrew Robinson, PhD 934-6421

Manager
Lisa Rice-Jennings 975-8367 or 975-8368

Outreach
Faculty
C. Bruce Alexander, MD 934-249 or 934-4303

Supervisor
Diane Hendricks 996-1900

Manager
Donna Scott 996-9698

Useful websites: The Standards and Clinical resources may only be available from computers on campus.

https://scr.hs.uab.edu/ useful to review test protocols (under Policies and Clinical Resources select UAB Medicine Labs then select Search and enter the test you are looking for.

http://www.labsource.hs.uab.edu/labsource/#/home useful for all reference ranges, sample collection, availability, panel content and other information under the Documents tab.

REFERENCE LABORATORIES: Information for reference laboratories and which tests we send where is located on the "L, resident info" drive in the resident offices. The Excel file is named "MSPRQIN"
If you open the file, the first tab is a listing of all the send-out tests, which also lists prices, TAT, requirements for consult, etc.
At the bottom of this page, there is another tab named "Referral Phone". If you click on this tab, it will show a listing of all the labs and their phone numbers. This also has UAB's client number for each lab, which is important to have handy if you have to call these labs for any reason. Ms. Donna Pirkle created this spreadsheet for the residents and updates it frequently with any changes.

If you have a question about how to use it she is very nice and receptive to questions, please contact her at 934-9585.

CHEMISTRY ROTATION

By the end of this rotation, the resident should have acquired:

1) An understanding of the basic technologies used by the Clinical Chemistry Laboratory.
2) An understanding of how to act as an expert consultant and liaison to clinicians.
3) A basic understanding of how the Clinical Chemistry Laboratory operates.
4) An understanding of quality control (QC) procedures and external proficiency testing.
5) An ability to evaluate the relevant literature in order to assess the need for and technical aspects of new laboratory tests, as they become available.

During the rotation, the resident will perform the following routine duties:

1) Sign out SPEP, and IFE
2) Review UAB send-out testing on proactive basis.
3) Weekly review of VA send-out tests [See Ina Walker].
4) Monthly review of QC results with faculty (Dr. Hardy).
5) Performing appropriate consults regarding send-out and other esoteric tests.
6) Performing (firsthand) prescribed complex manual or semi-manual laboratory assays as arranged by the faculty.
7) Giving a Laboratory Medicine Seminar (Lab Med Seminar) and presenting a journal club article on relevant Clinical Chemistry topic.

In addition to these activities, the resident will also be encouraged to work on a project by the faculty during the first week of the rotation. The goal of this project will be for the resident to help generate manuscript that can be submitted for publication.

The resident will attend the following conferences while on the rotation:

1) Resident Report, Monday Noon
2) Clinical Chemistry Section Meeting, Wednesday 9 a.m.
3) Experimental Pathology Seminar, Tuesday Noon
4) Laboratory Medicine Conference, Tuesday 11 a.m.
5) Endocrine Conference, Thursday Noon – BDB 334
6) Endocrine Case Conference, Tuesday 4:30 p.m. – 7th Floor BDB

Recommended Reading

General references: Quick Compendium of Clincal Pathology 3e: Chapters 1 and 8, this reference is very important to review during your rotation and again before board exams. The following chapters in Henry are suggested to supplement the syllabus that is handed out at the start of the rotation: 1-13, 14-29. For more detailed information, refer to the corresponding chapters in Tietz.

Journals: Clinical Chemistry, New England Journal of Medicine, JAMA, Lancet.

Specific References: Bakerman, Clinical Chemistry Review Notes, Wallach Interpretation of Diagnostic Tests, Wu, Cardiac Markers, Westgard, Basic QC practice and Basic Method Evaluation, Keren, Protein Electrophoresis in Clinical Diagnosis, Rifai, Handbook of Lipoprotein Testing.

Organization of the Chemistry Rotation:

The Chemistry Rotation is divided into 3 blocks (each one month long), as detailed below. During each block, the assigned faculty member will be responsible for giving the resident three 1-2 hour didactic sessions per week. It is, however, the resident's responsibility to arrange these teaching sessions in advance. The primary contact throughout will be Dr. Hardy. The resident should read the appropriate papers in the course syllabus, which provide an introduction to the primary literature and a review of important topics.

Month 1 (Dr. Hardy) – **Intro to Chemistry rotation.** Electrophoresis, quality control, lipids, lipoproteins, liver function testing, renal function testing, problem assays, water and electrolyte metabolism, acid-base disorders, cardiac markers, carbohydrates and diabetes mellitus.

Month 2 (Dr. Robinson) – General and forensic toxicology, drugs of abuse testing, alcohol analysis, ethylene glycol and analysis, drug screens by TLC, Toxilab procedure, GC/MS theory, therapeutic drug monitoring, point of care testing.

Month 3 (Dr. Smith) – General introduction to management, electrophoresis, enzymes, liver function testing, evaluation of endocrine function, including calcium and phosphate, amniotic fluid analysis.

Final evaluation at the end of the rotation will be based on the following:

1) Conference presentations, including the ability to read the relevant literature, extract important information, and make appropriate decisions.
2) Proficiency in interpretation of Clinical Chemistry tests.
3) Proficiency in interpretation of QC results.
4) Timeliness and thoroughness of performance of routine duties.
5) Attendance, punctuality, professional attitude, and interaction with professional and ancillary staff.
6) Special project involvement.

Laboratory Experience: The faculty member will also assign to the resident appropriate manual or semi-manual tests to perform that will demonstrate basic principles of Clinical Chemistry technology.

On-Call Responsibilities: The Chemistry Pager is important as it is the "one number" connection that is referred to on all our memos and provides a single contact point for physicians and staff to contact Chemistry. Your personal pager should also be carried as your name will be listed in the on call schedule.

Types of Calls to Expect: Approval of unusual tests, internal chemistry problems (lab, send out, customer service), Consultation questions from clinicians (these may take in depth investigation and can make interesting Case presentations at the Monday Noon Residents Report), physician alert of critical values.

Examples of Pager Calls:

a) Many Pager calls involve send-out test consults. When you receive the consult, look up the pt's history on CDA to get an idea of why the test is being ordered. Always try to call the ordering physician to make sure the test is still needed and to notify them of the TAT.

A good place to start when you receive a consult on a test you've never heard of:

- Bakerman's ABC's of Laboratory Data
- ARUP's Guide to Clinical Laboratory Testing (in some CP resident offices, also online at www.aruplab.com)
- Tietz Clinical Guide to Laboratory Testing
- DORA (Directory of Rare Analysis, can help you find a lab which measures unusual analytes not done by our normal reference labs)

Alcohol Profile and Ethylene Glycol Level Ordering/Approval Protocols
Prior approval based on patient's history and lab data is required for all orders on all patients by the resident or faculty. The test is a send out test except for serum ethanol which is done in house.

Method: Gas Chromatography

Protocol:

- Chemistry resident or faculty is contacted about order (from lab or clinical team)
- Resident or faculty discusses patient's history and laboratory findings with the clinical team and determines need for test (refer to algorithm). They should also be informed that it is a send out test except for the serum ethanol.
- *If not warranted, resident or faculty notifies clinical team and the lab and cancels the order
- If warranted, resident or faculty arranges for sample to be collected and the sample is sent out by Specimen Receiving (Diane Hendricks)

Sample requirements

- Minimum of 1 mL of serum is required
- Received in original tube
- DO NOT open tube (many alcohols are volatile)
- DO NOT use serum separator tubes
- DO NOT aliquot
- Keep sample refrigerated until sent out

***If conflicts arise with the clinical team or the laboratory staff, please page your (non-blood bank) faculty member on call or contact Dr. Hardy (see Chemistry Survival Guide for contact information).**

Commonly Encountered Lab Problems:

Glucose

Falsely decreased glucose values are often the product of non-analytical factors. Consider the following scenarios when evaluating situations of very low glucose values:

- Wrong tube type-should be grey-top NaF tube to prevent glycolysis
  - Old sample-especially if non-centrifuged at room temperature glucose will decrease 7 – 10%/hr in uncentrifuged samples at room temp.
  - Very high WBCs>500,000/mm^3 can drastically decrease glucose in less than 1 h only in non-centrifuged samples
  -

Another call concerns a request for approval for doing a glucose tolerance test on someone that has a fasting blood sugar >126 mg/dL (i.e. likely a diabetic). **The resident must get this approval from the attending physician.**

Electrolytes

Electrolyte concentrations can be affected by non-analytical factors such as sample collection and handling. Some commonly-encountered electrolyte issues are listed below:

- Decreased Na^+ in hyperglycemia – Na^+ can be estimated by the following calculation:

  *estimated Na^+ = Na^+ result + ((glucose- 100)*0.016)*
- Extremely low Ca^{2+}, Mg^{2+} (this also decreases ALP, CK) due to EDTA chelation via collection purple-top tube
- Increased K^+ due to collection in K_2EDTA (purple) tube or due to hemolysis
- Increased Li^+ due to collection in Li-heparin (green) tube
- Increased plasma K^+ (coupled with very low glucose) due to very high WBC

Immunoassays

A large number of tests performed in the laboratory utilize immunoassay methods. The three most common problems with these tests are listed below:

- Hook effect – observed with homogeneous, one-step immunoassay formats-(e.g. myoglobin, prolactin) very high concentrations of antigen result in inappropriately decreased values that may fail to dilute properly; to correct, dilutions of 1/100 or 1/1000 may be necessary

- Cross reactivity with structurally similar molecules-(e.g. cortisol) Serum cortisol will be inappropriately elevated in patients on certain corticosteroid therapies (see package insert), do urinary free cortisol
- Heterophilic antibodies (see heterophilic antibody interference section)

TDMs
It is important to know if the sample was drawn to determine a peak or trough drug level. Keep in mind that digoxin, lithium, and cyclosporin distribute into the body very slowly, therefore, serum samples drawn before distribution will yield elevated values.

Restricted Test Time:
Routine Chemistry (Core) Lab 24x7

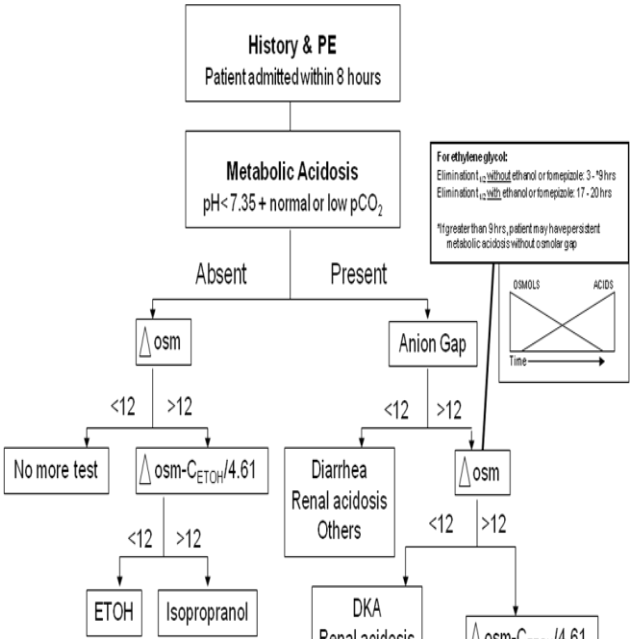
Special Chemistry Lab 7:00-4:30 M-F, 7:00-3:30 S & S (as necessary 2nd or 3rd shift might be asked to run a stat special chemistry test, i.e. TSH/FT4)

Chain of Custody Issues: Any case involving medical/legal implications should be collected for law enforcement and taken to the proper agency. UAB's tests are for medical use only and are presumptive positive only. If a chain of custody/legal issue arises day or night, notify your Lab Medicine attending. They may suggest you talk to the Risk Management attorney who is on call.

Ethylene Glycol/Alcohol Profile Requests: The clinician should be notified that these are send-out tests with the exception of serum ethanol which is done in house.

When comparing the measured osmolality with the calculated osmolality, make sure the sample being used to measure osmolality is from around the same time as the fluid balance profile you are using to calculate the osmolality. If they are from different time frames, ask the lab to run another fluid balance profile from the sample they received to measure the osmolality. The following Table and Flow Chart will help you with this process.

NOTE: Once an alcohol profile or ethylene glycol has been approved, the Special Chemistry Lab must be notified.



1. Anion Gap = C_{Na} (mEq/l) - [C_{HCO3} (mEq/l) + C_{Cl} (mEq/l)]
2. Δ osm (mosm/kg) = Measured Osmolarity - Calculated Osmolarity
   Calculated Osmolarity = 2 x C_{Na} (mEq/L) + $C_{glucose}$ (mg/dl)/18 + UREA* (mg/dl)/2.8
   *UREA (mg/dL) = BUN (mg/dL) X 2.14
3. C_{ETOH}: Serum level of ethyl alcohol (mg/dl)

For half-life of other alcohols, refer to following page.

Alcohol Half-Life

Ethylene Glycol:

- *3-9 hours*
- *~18 hours with a blood ethanol of 100 mg/dL; a similar increase in half-life is seen with fomepizole treatment*
    - *Note: the two do not appear to be additive with respect to increasing the half-life of ethylene glycol--still ~18 hours with both on board*

Methanol:

- *14-20 hours: mild intoxication*
- *24-30 hours: severe intoxication*
- *31-52 hours with ethanol therapy*
- *22-87 hours with fomepizole therapy*
- *3.5 hours with ethanol and hemodialysis therapies*
- *Metabolite – formate has a half-life of 2-4 hours (up to 20 hours with fomepizole therapy)*

Isopropanol:

- *2-8 hours*
- *Prolonged by ethanol*
- *Metabolite – acetone has a half-life of 17-27 hours*

TOXICITY OF ETHANOL, METHANOL, ISOPROPYL ALCOHOL, AND ETHYLENE GLYCOL

Compound	Toxic Metabolite(s)	Approximate Lethal Dose (mL/kg)	Symptoms & Signs of Poisoning
Ethanol		3-4	Drunkenness: disinhibition, ataxia, slurred speech; hypoglycemia; stupor and coma; respiratory arrest
Ethylene glycol	Oxalic, glycolic hippuric accids	1.5	Parent compound: same as ethanol. Metabolites: metabolic acidosis; hypocalcemia; calcium oxalate crystalluria; acute renal failure; cardiac conduction disturbances; seizures
Isopropyl alcohol	Acetone	2-3	Effects are identical to ethanol, although usually about twice as potent. Gastritis is common after ingestion of 70% rubbing alcohol
Methanol	Formic acid	1-2	Parent compound: same as ethanol. Formic acid: metabolic acidosis; severe visual disturbances leading to blindness; coma; seizures; respiratory arrest

Sendout Holiday Tests:

Herpes SV PCR, CSF
Mycobacteria, B DNA CSF PCR (tuberculosis)
Immuknow

Serotonin Release Assay HIT
Von Willebrands Protease Activity

Bacterial Antigen, LPA CSF/Urine
E. coli antigen CSF
H influenza B antigen
N meningitis Group B/E coli K1 antigen
Pneumococcal antigen, pediatric
Rotavirus antigens
Streptococcus, Gp B CSF

Cholinesterase, RBC and plasma
L/S ratio
Nitroprusside Profile (cyanide, thiocyanate)
Phosphatidylglycerol
Procainamide NAPA
Pseudocolinesterase, serum

Suspected Heterophilic Antibody Interference (aka HAMA interference):

Many assays performed in the lab utilize animal antibodies in a sandwich format. The presence of endogenous anti-animal immunoglobulin (Ig) antibodies in patient sera can cause erroneous results in such immunoassays. Falsely elevated results due to heterophile antibody interference are now quite rare but have been reported for CEA, CA-125, TSH, AFP, cardiac Troponin I and h CG. This problem may manifest itself as an inappropriately elevated result or as an inconsistency between two related immunoassays (e.g., T_4 and TSH). You should consult with a medical director on such questions. Actions for detecting a heterophile antibody interference include: analyzing the sample by a different method, on dilution, and with HAMA blocking reagent, and repeating serum tests on urine (hCG). Also, check the patient record for other inappropriate elevations in immunoassay results.

Recording & Reporting Format:

Please follow these guidelines.

- Be concise. **Be organized and do not ramble.**
- Present pertinent background on patient. State the problem and/or question.
- Discuss how the call/problem/question was resolved.
- For patient cases, be sure to provide actual lab results on the patient.
- Include patient hospital number when available.
- Discuss follow-up to the problem.
- It is imperative to follow-up on cases presented,
i.e., ensure that proper corrective action was taken or information relayed. This is most often done at the following weeks' beeper report. Is the result consistent with the patient's condition? Have you contacted the appropriate person to set up a new policy to prevent

similar incidents from happening again? If from lab personnel, did you follow-up and report what happened?

Physician Alert Values & How to Report Them:

One area where it's hard to find the ordering physician is the ER. By the time the lab notifies you of the result, the doctor has gone home from his/her shift. Try the physician first, as he/she may respond to your page. If he/she doesn't respond, call the ER and speak to the charge nurse. The charge nurse can then get in touch with the correct people.

If the attending/on-call person cannot be reached the UAB operator can directly call his/her home number. As an absolute last resort the patient can be contacted directly.

Chemistry Journal Club Presentation:

The idea for this presentation is to drill down in depth on one paper and go into great detail on the complete paper, including statistics, methodology and all data presented. Papers must be clearly related to Clinical Chemistry.

Analyte Stability Information:

CHEMISTRY ANALYTE STABILITY CHART									
CHEMISTRY POLICY: CP.06.16	ISSUE DATE: 19-Apr-99			REVIEWED DATE: 23-May-02					
	UNPROCESSED SPECIMENS		ON GEL (PROCESSED)			SEPARATED FROM CELLS			
	Room Temp	4° C	-20° C	Room Temp		4° C	Room Temp	4° C	-20°C
ACE	2 Hr	2 Hr	N/A	2 Hr		2 Hr	2 Hr	4 Hr	Do Not Freeze
Acid Phosphatase	1 Hr	2 Hr	N/A	2 Hr		8 Hr	2 D @ pH 5.0	8 D @ pH5	4 Mo @ pH 5
AFP	24 Hr	24 Hr	N/A	24 Hr		24 Hr	24 Hr	24 Hr	1 Mo
Albumin	2 D	4 D	N/A	4 D		6 D	1 Wk	1 Mo	> 3 Mo
Aldolase	2 Hr	2 Hr	N/A	2 Hr		2 D	2 D	3 D	15 D
Aldosterone	24 Hr	24 Hr	N/A	24 Hr		4 D	2 D	4 D	1 Wk
Alkaline Phosphatase	24 Hr	24 Hr	N/A	24 Hr		2 D	24 Hr	4 D	2 Mo
ALT	24 Hr	24 Hr	N/A	24 Hr		1 Wk	24 Hr	1 Wk	2 D
Ammonia (On ICE)	15 Min	30 Min	N/A	15 Min		30 Min	15 Min	30 Min	2 D
Amylase	2 D	4 D	N/A	4 D		4 D	1 Wk	1 Wk	6 Mo
Amylase, Urine	2 D	1 Wk	3 Wk						
AST	24 Hr	24 Hr	N/A	24 Hr		2 D	2 D	4 D	3 Mo
Bicarbonate (tCO_2)	4 Hr	4 Hr	4 Hr	8 Hr Closed		12 Hr Tube	2 Hr Closed	2 Hr Tube	N/A
Bilirubin, Direct (In Dark)	4 Hr	4 Hr	N/A	24 Hr		2 D	12 Hr	4 D	2 Wk
Bilirubin, Total (In Dark)	4 Hr	4 Hr	N/A	24 Hr		2 D	12 Hr	4 D	2 Wk
Blood Gas pH	↓0.01/1 Hr @ 37° C								
pCO_2	↑ 0.7 mmHg/ 1 Hr								
$p0_2$	1 Hr								
CA 125	24 Hr	24 Hr	N/A	24 Hr	5 D	24 Hr	5 D	3 Mo	
CA 15-3	12 Hr	24 Hr	N/A	12 Hr	5 D	12 Hr	5 D	3 Mo	
Calcium	2 D	2 D	N/A	2 D	4 D	1 Wk	10 D	> 8 Mo	
Calcium, Ionized (Closed Container)	2 Hr	4 Hr	N/A	4 Hr	8 Hr		4 Hr		
Calcium,Urine (pH<3.0)	2 D	4 D	6 Mo						
CEA	24 Hr	24 Hr	N/A	24 Hr	5 D	24 Hr	5 D	6 Mo	
Chloride	24 Hr	2 D	N/A	2 D	4 D	1 Wk	1 Wk	>1 Yr	
Chloride, Urine	4 D	1 Wk	> 1 Yr						
Cholesterol	2 D	2 D	N/A	2 D	4 D	1 Wk	1 Wk	3 Mo	
CPK	24 Hr	2 D	N/A	24 Hr	4 D	2 D	1 Wk	1 Mo	
CK-MB	12 Hr	24 Hr	N/A	24 Hr	4 D	24 Hr	24 Hr	1 Mo	
Cortisol	24 Hr	24 Hr	N/A	2 D	5 D	3 D	1 Wk	> 3 Mo	
Creatinine	24 Hr	24 Hr	N/A	4 D	4 D	1 Wk	1 Wk	3 Mo	
Creatinine, Urine	2 D	6 D	6 Mo						
Digoxin	24 Hr	24 Hr	N/A	24 Hr	5 D	2 D	1 Wk	6 Mo	
Estradiol	24 Hr	2 D	N/A	24 Hr	3 D	24 Hr	3 D	1 Yr	
Ferritin	12 Hr		N/A	3D	3D	2D	3D	3D	

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	UNPROCESSED SPECIMENS		**ON GEL (PROCESSED)**			**SEPARATED FROM CELLS**			
	Room Temp	**4° C**	**-20° C**	**Room Temp**		**4° C**	**Room Temp**	**4° C**	**-20°C**
Folate, Plasma	3 Hr		N/A	3 Hr	6 Hr	3 Hr	6 Hr	8 Wk	
Folate, RBC	12 Hr	2 D	4 D						
FSH	12 Hr	12 Hr	N/A	24 Hr	1 Wk	1 Wk	1 Wk	1 Yr	
Gamma-GT	24 Hr	2 D	N/A	24 Hr	4 D	1 Wk	1 Wk	Indef.	
Glucose	<2 Hr	3 Hr	N/A	2 Hr	4 Hr	24 Hr	1 Wk	4 Mo	
Glucose (Fluoride)	4 D	4 D	N/A			24 Hr	1 Wk	4 Mo	
Glucose, CSF	2 Hr	4 D (Spun)	2 Mo (Spun)						
Glucose, Urine	2 Hr	24 Hr	> 2 D						
Glycated Hemoglobin	2 D	1 Wk	6 Mo						
HCG, Serum	24 Hr	24 Hr	N/A	24 Hr	3 D	24 Hr	3 D	1 Yr	
HDL-Cholesterol	2 D	5 D	N/A	2 D	5 D	2 D	1 Wk	4 Mo	
Hemoglobin Saturation									
Oxyhemoglobin	1 Hr								
Carboxyhemoglobin	1 Hr	24 Hr							
Methemoglobin	1 Hr	2 Hr							
Iron	6 Hr	24 Hr	N/A	12 Hr	2 D	4 D	1 Wk	1 Yr	
Iron Binding Capacity	6 Hr	24 Hr	N/A	12 Hr	2 D	4 D	1 Wk	1 Yr	
Lactate (Fluoride)	2 Hr	4 Hr	N/A			3 D	3 D	N/A	

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	Room Temp	**4° C**	**-20° C**	**Room Temp**		**4° C**	**Room Temp**	**4° C**	**-20°C**
LDH	3 Hr	4 Hr	N/A	4 Hr	24 Hr	1 Wk	2 D	Do Not Freeze	
LH	12 Hr	12 Hr	N/A	24 Hr	1 Wk	1 Wk	1 Wk	1 Yr	
Lipase	24 Hr	24 Hr	N/A	24 Hr	4 D	1 Wk	1 Wk	1 Yr	
Magnesium	2 D	2 D	N/A	2 D	5 D	1 Wk	1 Wk	1 Yr	
Magnesium, Urine	2 D	3 D	1 Yr						
Myoglobin	2 Hr	3 Hr	N/A	12 Hr	2 D	2 D	1 Wk	3 Mo	
5' Nucleotidase	6 Hr	12 Hr	N/A	12 Hr	2 D	24 Hr	4 D	18 Wk	
Osmolality (Closed Tube)	6 Hr	12 Hr	N/A	8 Hr	24 Hr	6 Hr	24 Hr	3 Mo	
Phosphorus	3 Hr	4Hr	N/A	24Hr	2D	3D	1 Wk	1 Yr	
Phosphorus, Urine (pH<5.0)	2 D	1 Wk	6 Mo						
Potassium	4 Hr	1 Hr	N/A	12 Hr	12 Hr	4 D	1 Wk	1 Yr	
Potassium, Urine	2 D	8 D	1 Yr						
Progesterone	12 Hr	24 Hr	N/A	12 Hr	24 Hr	12 Hr	3 D	1 Yr	
Prolactin	1 D	1 D	N/A	2 D	5 D	2 D	5 D	1 Yr	
Protein, CSF	24 Hr	4 D	3 Mo						
Protein, Total	24 Hr	24 Hr	N/A	2 D	4 D	6 D	4 Wk	1 Yr	
Protein, Urine	24 Hr	3 D	1 Mo						
PSA	24 Hr		N/A	3 D	5 D	24 Hr	1 Wk	3 Mo	
Sodium	2 D	3 D	N/A	4 D	1 Wk	2 Wk	2 Wk	1 Yr	
Sodium, Urine	2 D	1 Wk	1 Yr						
Testosterone	24 Hr	2 D	N/A	24 Hr	3 D	24 Hr	3 D	1 Yr	
T4, Free	12 Hr	12 Hr	N/A	24 Hr	24 Hr	24 Hr	2 D	>1 Mo	
Thyroxine (T4)	24 Hr	2 D	N/A	2 D	1 Wk	2 D	1 Wk	> 1 Mo	
T-Uptake	24 Hr	24 Hr	N/A	24 Hr	2 D	2 D	1 Wk	> 1 Mo	
Total T3	24 Hr	2 D	N/A	2 D	1 Wk	2 D	1 Wk	> 3 Mo	
TSH	24 Hr	24 Hr	N/A	24 Hr	1 Wk	2 D	1 Wk	3 Mo	
Triglyceride	2 D	3 D	N/A	2 D	5 D	2 D	1 Wk	1 Yr	

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	UNPROCESSED SPECIMENS		ON GEL (PROCESSED)			SEPARATED FROM CELLS		
	Room Temp	4° C	-20° C	Room Temp		4° C	Room Temp	4° C	-20°C
Troponin I	12 Hr	2 D	N/A	2 D	2 Wk	2 D	1 Wk	1 Yr	
Urea	24 Hr	2 D	N/A	2 D	4 D	1 Wk	1 Wk	1 Yr	
Urea, Urine	2 D	1 Wk	1 Mo						
Uric Acid	24 Hr	2 D	N/A	2 D	4 D	2 D	1 Wk	2 Mo	
Uric Acid, Urine	4 D	4 D	4 D						
Vitamin B12	6 Hr	6 Hr	N/A	6 Hr	6 Hr	6 Hr	6 Hr	8 Wk	

Full Draw Vacutainer(tm) Tubes			
Tubes with Hemogard(tm) Closure	**Tubes with Conventional Stopper**	**Additive**	**Laboratory Use**
 Gold	 Red/Black	• Clot activator and gel for serum separation	• Serum Separator Tube for serum determinations in chemistry. Tube inversions ensure mixing of clot activator with blood. Blood clotting time 30 minutes.
 Light Green	 Green/Gray	• Lithium heparin and gel for plasma separation	• BD Vacutainer(tm) PST(tm) Tube for plasma determinations in chemistry. Tube inversions prevent clotting.
 Red	 Red	• None (glass) • Clot activator (plastic tube with BD Hemogard(tm) closure)	• For serum determinations in chemistry and serology. Glass serum tubes are recommended for blood banking. Plastic tubes contain clot activator and are **not** recommended for blood banking. Tube inversions ensure mixing of clot activator with blood and clotting within 60 minutes.
 Orange	 Gray/Yellow	• Thrombin	• For stat serum determinations in chemistry. Tube inversions ensure complete clotting which usually occurs in less than 5 minutes.
 Royal Blue		• Sodium heparin • Na_2EDTA • None (serum tube)	• For trace-element, toxicology and nutritional-chemistry determinations. Special stopper formulation provides low levels of trace elements (see package insert).

Full Draw Vacutainer(tm) Tubes			
Tubes with Hemogard(tm) Closure	**Tubes with Conventional Stopper**	**Additive**	**Laboratory Use**
		• Sodium heparin	• For plasma determinations in chemistry. Tube inversions prevent clotting.
Green	Green	• Lithium heparin	
		• Potassium oxalate/sodium fluoride	• For glucose determinations. Oxalate and EDTA anticoagulants will give plasma samples. Sodium fluoride is the antiglycolytic agent. Tube inversions ensure proper mixing of additive and blood.
Gray	Gray	• Sodium fluoride/Na_2 EDTA	
		• Sodium fluoride (serum tube)	
		• Sodium heparin (glass)	• For lead determinations. This tube is certified to contain less than .01µg/mL(ppm) lead. Tube inversions prevent clotting.
Tan		• K_2EDTA (plastic)	

Partial Draw and Pediatric Vacutainer(tm) Tubes			
Partial-draw Tubes (2ml and 3ml: 13 x 75mm)	**Small-volume Pediatric Tubes (2ml: 10.25 x 64mm)**	**Additive**	**Laboratory Use**
		• None	• For serum determinations in chemistry and serology. Glass serum tubes are recommended for blood banking. Plastic tubes contain clot activator and are **not** recommended for blood banking. Tube inversions ensure mixing of clot activator with blood and clotting within 60 minutes.
Red	Red		
		• Sodium heparin	• For plasma determinations in chemistry. Tube inversions prevent clotting.
Green	Green	• Lithium heparin	

Urine Drug Screening:
Urine is the sample of choice for drug screening because metabolites are excreted in the urine. Thus the sensitivity is increased. The UAB emergency laboratory screens for the following amphetamines, opiates, cocaine metabolite (benzoyleconine), barbiturates, benzodiazepines, cannabinoids, 6Monoacetylmorphine, oxycodone, methadone, and buprenorphine.

These are enzyme immunoassays, as such they are screening tests and do not indicate the person is under the influence at the time of assay.

Amphetamines, this is used to determine if the person has used D-amphetamine, D-methamphetamine, Methylenedioxyamphetamine, or methylenedioxymethamphetamine. False positives may be found with therapeutic doses of chloropromazine, methoxyphenamine, rantidine, isometheptine, procainamide and NAPA. High concentrations of ephedrine, phenylpropanolamine or pseudoepheridine may cause a false positive.

Opiates, this test is designed to determine if a person has used morphine, codeine hydrocodone, or heroin. A positive result indicates the presence of opiates but does not indicate or measure intoxication. Meperidine and nalorphine cause false positives. Oxycodone, trazadone, and methadone are not opiates and usually do not react with the opiate antibody.

6 Monoacetylmorphine, is metabolite of heroin and indicates heroin use.

Benzoylecogonine, this is used to determine if this person has used cocaine within the past 72 hours. A positive result does not indicate intoxication, but only use within the past 72 hours. If the case is medical/legal a blood sample must be collected in a NaF tube for cocaine analysis. Cocaine analysis is a send out test.

Benzodiazepines are sedative-hypnotic drugs which include chlordiazepoxide, diazepam, oxazepam, and alprazolam. Benzodiazepines potentiate the effect of ethyl alcohol. A positive result indicates use of a benzodiazepine within the past 72 hours, but does not indicate intoxication. A number of compounds interfere including citalopram and oxaprozin.

Barbiturates are central nervous and respiratory system depressants and are classified based on their duration of action. The test will be positive following the use of Phenobarbital, amobarbital, butabarbital, pentobarbital, secobarbital. High concentrations of phenytoin or thiopental will also be positive.

Oxycodone is a semisynthetic analgesic used to treat pain.

Methadone is a synthetic opiod used to treat pain and used in detoxification in narcotic addiction.

Buprenorphine is a synthetic opiod used in the treatment of opiod dependence.

Cannabinoids are the components in marijuana which produce the hallucinogenic effects. A positive result indicates the use of marijuana within the past 21 days but does not indicate intoxication. False positives may result from the use of pantoprazole.
Any case involving medical/legal implications should be collected for law enforcement and taken to the proper agency. UAB's tests are for medical use only and are presumptive positive only.

Protein Electrophoresis in Serum and Urine: Techniques and Interpretation

John D. Pfeifer, M.D., Ph.D., Robin G. Lorenz, M.D., Ph.D.

The use of electrophoresis to separate biological fluids into their component protein bands was first introduced more than 50 years ago. Electrophoresis is a method of separating charged molecules on the basis of their relative mobility in an electric field. The direction of migration of a protein in an electric field depends on the pH of the buffer and the isoelectric point of the protein. Electrophoresis of protein-containing samples of an agarose film at pH 8.6 separates the proteins into bands that occur in five well-defined regions.[3] Following electrophoresis, the separated protein bands are fixed in place and visualized by staining with Amido Black. The most common use of electrophoresis today is to detect **monoclonal** immunoglobulins in serum or urine, which can provide confirmatory evidence of malignancy. However, as will be described in this newsletter, electrophoresis can also be used as a screening test to provide information about a patient's nutritional, hepatic, renal, and inflammatory status.

Serum Electrophoresis – Standard agarose gel electrophoresis systems separate serum into five zones designated albumin, alpha-1, alpha-2, beta, and gamma (Fig. 1). The protein content of each is calculated after measuring the total serum protein and integrating the densitometric scan of the gel. The stained gel is then visually carefully inspected to detect qualitative abnormalities.[48] Thirteen proteins represent > 90% of the total protein mass of serum, and information about most of these is obtained by serum protein electrophoresis (SPEP) on agarose.[3]

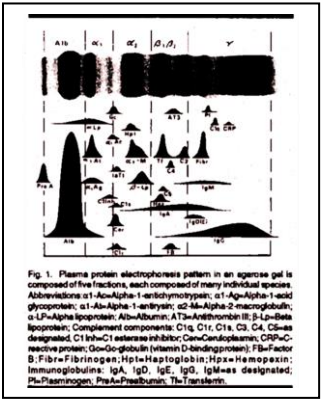

Fig. 1. Plasma protein electrophoresis pattern in an agarose gel is composed of five fractions, each composed of many individual species. Abbreviations: α1-Ac=Alpha-1-antichymotrypsin; α1-Ag=Alpha-1-acid glycoprotein; α1-At=Alpha-1-antitrypsin; α2-M=Alpha-2-macroglobulin; α-LP=Alpha lipoprotein; Alb=Albumin; AT3=Antithrombin III; β-Lp=Beta lipoprotein; Complement components: C1q, C1r, C1s, C3, C4. CS=as designated, C1 Inh=C1 esterase inhibitor; Cer=Ceruloplasmin; CRP=C-reactive protein; Gc=Gc-globulin (vitamin D-binding protein); FB=Factor B; Fibr=Fibrinogen; Hpt=Haptoglobin; Hpx=Hemopexin; Immunoglobulins: IgA, IgD, IgE, IgG, IgM=as designated; Pl=Plasminogen; PreA=Prealbumin; Tf=Transferrin.

Albumin is the most prominent band on a normal SPEP, and decreased albumin is the most common abnormality seen on this band. Chronic inflammatory conditions are associated with modest hypoalbuminemia, while more severe depressions occur with protein-losing syndromes such as severe malabsorption, glomerulonephritis, protein-losing enteropathies, burns, and severe acquired liver disease (Fig. 2).

The alpha-1 zone contains mostly α-antitrypsin with minor contributions from other proteins (Fig. 1). A marked decrease in the alpha-1 band may suggest α1-antitrypsin deficiency and should prompt further investigation, as low levels predispose to early-onset emphysema. Serum α1-antitrypsin levels are measured in the clinical immunology laboratory by nephelometry, and decreased levels are confirmed by determining the phenotype through electrofocusing.[2] α1-Antitrypsin is an acute phase reactant whose levels increase in response to inflammation and tissue necrosis.

The principal components of the alpha-2 zone are α2-macroglobulin and haptoglobin. Marked elevation of α2-macroglobulin is seen in the nephrotic syndrome. Decreased levels of both α2-macroglobulin and haptoglobin may be seen in acquired liver disorders. Haptoglobin levels increase in response to inflammation and tissue necrosis (Fig. 2) One common SPEP artifact is seen

when the serum sample is hemolyzed. When *in vitro* hemolysis occurs, hemoglobin-haptoglobin complexes form and migrate in a broad band between the alpha-2 and beta globulin regions. Although this is easily mistaken for a monoclonal protein, the correct interpretation can be made by visual inspection of the sample for hemolysis or by immunofixation.

The beta globulin zone contains three major protein components; transferrin, the third component of complement (C3), and ß-lipoprotein (LDL). The C3 band is quite labile and is often absent unless fresh serum is used. A more reliable measure of C3 levels can be obtained by nephelometry, which is performed in the Clinical Immunology Laboratory. LDL appears as a wavy band that can increase in intensity in a number of conditions, including liver diseases, nephrotic syndromes, and some familial hyperlipidemia syndromes. Transferrin is elevated in iron deficiency anemia and decreased during the acute-phase response and by severe acquired liver disease (Fig. 2). The elevation of transferrin can easily be mistaken for a monoclonal immunoglobulin abnormality. This can be clarified by immunofixation electrophoresis. [9]

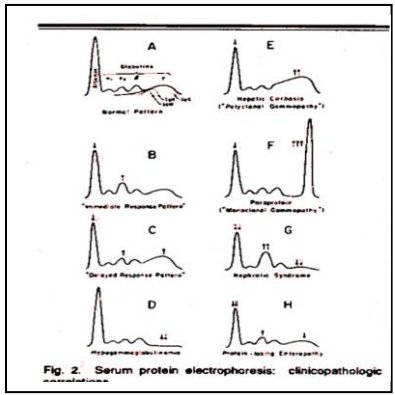

Fig. 2. Serum protein electrophoresis: clinicopathologic

The majority of immunoglobulins are found in the gamma globulin zone. This heterogeneous group of proteins is distributed throughout the entire region, and they do not ordinarily produce discrete bands. The finding of a band in the gamma region should alert the physician to the possibility of a monoclonal paraprotein (Fig. 2). The presence of a monoclonal paraprotein is confirmed by the more sensitive immunofixation electrophoresis, where the exact heavy and light chains of the immunoglobulin can be determined. Other proteins that may mimic the pattern of a monoclonal paraprotein are C-reactive protein, lysozyme, and traces of fibrinogen. A discrete band of C-reactive protein may be seen in the acute phase response, however the presence of discrete bands of lysozyme and

fibrinogen in some clinical samples currently has no known clinical significance.

The most important use of serum protein electrophoreses is for the detection of monoclonal paraproteins.[6] It should be noted that the immunoglobulins are not confined to the gamma globulin fraction. Most serum IgA is found in the alpha-2 and beta fractions, and small amounts of IgM and IgG are also present in these fractions. Identification of monoclonal serum immunoglobulin is suggestive of a B-cell malignancy, particularly in concert with other clinical and laboratory evidence of multiple myeloma or B-cell lymphoma. The electrophoretic techniques currently available can detect normal as well as malignant clonally restricted proliferations of B cells, so the clinical pathologist's interpretation must be integrated with the findings of other test results in order for the correct diagnosis to be determined. Following diagnosis, monitoring serum levels of the paraprotein may serve as a useful measure of tumor burden, while identification of monoclonal immunoglobulin fragments in urine may be of prognostic significance.[7]

Quantitative abnormalities in immunoglobulins can also be detected by SPEP. Hypogammaglobulinemia is seen as a decreased staining intensity which may be symmetrical or asymmetrical. If the densitometer scan of the gamma region does not approximate a symmetrical, bell-shaped curve, then there may be an underlying isotype imbalance. This can be confirmed by IgG, IgM, and IgA quantitation, as well as IgG subclass determination. Marked hypogammaglobulinemia in an older individual, with no indication of qualitative serum immunoglobulin abnormalities, should be followed up with a urine protein electrophoresis to screen for monoclonal free light chains. Hypergammaglobulinemia can be due to polyclonal, oligoclonal, or monoclonal gammopathies. True polyclonal increases are seen in patients with severe liver disease due to the altered blood flow through the liver, which shunts intestinally derived antigens to the peripheral tissues where an increased immune response can now occur. Polyclonal increases with an oligoclonal component are common in patients with connective tissue diseases.

In addition to abnormalities described above, certain diseases produce alterations in more than one protein and therefore affect the overall electrophoretic pattern. These patterns may be recognized on visual examination of the gel, or by quantitative densitometric scanning (Fig. 2). The Clinical Immunology Laboratory will begin reporting out patterns consistent with several diseases early in 1995 (Table I).

Urine Electrophoresis – Urine protein electrophoresis (UPEP) is indicated during the workup of any patient with proteinuria. It should be noted that urine dipstick tests for protein detect mainly albumin and may grossly underestimate amounts of other proteins present, such as monoclonal free light chains (Bence-Jones proteins). UPEP is useful for differentiating glomerular from tubulointerstitial proteinuria, and when combined with more sensitive urine immunofixation electrophoresis, can detect and characterize monoclonal immunoglobulins.

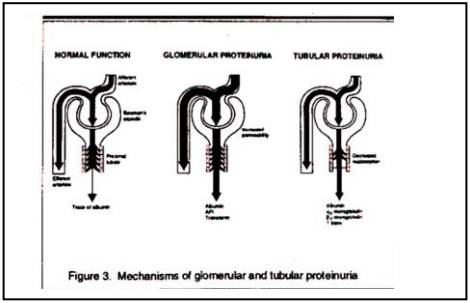

Figure 3. Mechanisms of glomerular and tubular proteinuria

Monoclonal free light chains are frequently synthesized in excess by the malignant plasma cells of multiple myeloma patients and may play a role in the development of secondary renal disease or amyloidosis. The presence of Bence-Jones proteinuria in patients with multiple myeloma usually indicates a poorer prognosis.[7] Quantitative measurement of the daily excretion of Bence-Jones protein provides an objective index of response to therapy. This has classically been performed on 24-hour urine specimens; however, a recent study has concluded that an early morning specimen is equivalent or slightly better for the detection and monitoring of light chain proteinuria.[1]

The actual control of urinary protein filtration is complex, however it is known that the ability of plasma proteins to traverse the glomerular basement membrane is directly correlated with their serum concentration and inversely correlated with their molecular size. Glomerular proteinuria is characterized by the passage of high-molecular-weight plasma proteins into the urine (Fig. 3). Albumin usually constitutes the bulk of the protein loss, however transferrin, α1-antitrypsin and IgG are also frequently detected. The hallmark of tubular proteinuria is the increased excretion of proteins of small size and an albumin content of 10-20%. These proteins

appear on UPEP as a heterogeneous group of bands, mostly of alpha-2 and beta mobility. Note that although these descriptions are of the patterns of pure glomerular or tubulointerstitial proteinuria, some UPEPs performed will exhibit features of both. Beginning early in 1995, the Clinical Immunology Laboratory will begin reporting out these patterns (Table I), in addition to the current reporting of the presence or absence of possible monoclonal immunoglobulins.

Clinical indications for the evaluation of immunoglobulins by protein electrophoresis - The presence of several laboratory criteria may alert the physician to the possibility of an immunoglobulin abnormality. These include an increased ESR, elevated total serum protein, proteinuria, and hypercalcemia. In addition, the following clinical signs may also indicate the need for SPEP or UPEP. The most important are chronic normochromic normocytic anemia of unknown etiology, nephrotic syndrome in a nondiabetic older patient, osteolytic lesions, lymphadenopathy, transient ischemic attacks, and systemic infections. The malignant diseases associated with a serum monoclonal gammopathy include multiple myeloma, B-cell lymphoma (including Waldenström's macroglobulinemia), heavy-chain disease, and amyloidosis. It should be noted that patients without a detectable immunoproliferative process may also present with monoclonal proteins in the serum. This has been termed monoclonal gammopathy of undetermined significance (MGUS). Patients with MGUS should be observed indefinitely, as studies have shown that approximately 20% of these patients will go on to develop a malignant disease within 10 years.[5]

Table 1. Common Protein Electrophoresis Patterns
Serum
Acute Inflammatory Disorder
Chronic Inflammatory Disorder
Nephrotic Syndrome
Hepatic Cirrhosis
Severe Hepatic Dysfunction
Hypoproteinemia
Urine
Glomerular Proteinuria
Tubular Proteinuria
Mixed Glomerular and Tubular Proteinuria

Test Availability – The Chemistry Laboratory performs serum and urine protein and immunofixation electrophoresis five days/week (M-F) with the results available the late in the afternoon. The recommended random sample for urine electrophoresis is the early morning specimen. All electrophoretic tests are reviewed by laboratory medicine residents and a medical director between 2:00 and 4:00 PM each weekday.

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Hints and Tips for Evaluating Electrophoresis Gels

1. First evaluate the overall quality of the gel:
   - For serum/urine gels check to make sure that you can easily differentiate all 5 regions (albumin; alpha-1; alpha-2; beta, gamma) and can see the paraprotein in the abnormal control
   - For CSF gels, make sure that there are paired serum and CSF samples and that you can see the prealbumin and end gamma-trace band

2. Next evaluate each of the 5 regions in all patients and controls, looking specifically for gross increases or decreases in each region.

3. Then carefully look at each patient, focusing on the gamma and beta regions.
   - If the patient has an abnormal band, first check to see if the patient is known to us.

   - If the patient has had an electrophoresis before, then compare today's test with their previous pattern. If it is similar, then comment that and record the information on the patient's card. Nothing further needs to be done.

   - If the patient has had an electrophoresis before, and today's pattern is different, then comment that, record the information on the card, and order an immunofixation to evaluate the changes.

- If the patient is new to us, order an immunofixation to get more information on the paraprotein and make a new file card with all their information.

4. When evaluating immunofixes, here are some general guidelines:
   - Free light chains are common in the urine, but not in the serum. If you see a free light chain in the serum on a new patient, always repeat the light chain with D and E heavy chains. This is not necessary in the urine.

   - Always make sure that the paraprotein seen on the immunofixation correlates with abnormal band seen on the electrophoresis.

5. Guidelines for positive bands on CSF electrophoresis:
   - CSF Isoelectric focusing gels are called positive if there are three or more bands seen in the CSF that are not seen in the serum.

   - CSF High Resolution Electrophoretic gels are called positive if there are two or more bands seen in the CSF that are not seen in the serum.

   - If there is no paired serum sample, and there are a significant number of bands in the immunoglobulin region on the gel, then the patient is reported as positive with the comment that the significance of the results are unknown due to lack of a paired serum sample.

Analysis of Cryoglobulins

Robin G. Lorenz, M.D., Ph.D., and John D. Pfeifer, M.D., Ph.D.

Introduction - Immunoglobulins that reversibly precipitate in the cold were first described in 1933 by Wintrobe and Buell.1 The term "cryoglobulin" (cold precipitable serum globulin) was suggested in 1947 and has since become common usage.2 While low levels of cryoglobulins have been detected in almost 40% of healthy controls,3,4 levels are markedly elevated in many disease states. The clinical usefulness of cryoglobulin analysis resides in its use as an aid in diagnosis and monitoring disease activity. Other cold-insoluble serum proteins that need to be distinguished from cryoglobulins include cryofibrinogens (which precipitate through an interaction with plasma fibronectin), complexes of C-reactive protein and albumin, and heparin precipitable proteins.5

Cryoglobulins have been found in association with multiple myeloma and Waldenstrom's macroglobulinemia, chronic infections, auto-immune diseases, acute and chronic liver diseases, acute and chronic glomerulonephritis, and in patients for which no underlying disease can be identified (termed essential cryoglobulinemia). A more complete list of the clinical associations of cryoglobulinemia is shown in Table I.

Table I - Diseases Associated with Cryoglobulinemia*

A. Infection
Viral:

B. Cytomegalovirus
Hepatitis B (acute and chronic)
Hepatitis C
Adenovirus
Bacterial:
Subacute bacterial endocarditis
Lepromatous leprosy
Acute poststreptococcal nephritis
Syphilis
Fungal:
Coccidioidomycosis

Parasitic:
Toxoplasmosis
Echinococcosis
Malaria
Schistosomiasis

C. Autoimmune Disease
Systemic lupus erythematosus
Rheumatoid arthritis
Polyarteritis nodosa
Sjögren's syndrome
Scleroderma (Raynaud's phenomenon)
Behcet's syndrome
Thyroiditis
Waldenström's macroglobulinemia

Multiple myeloma
Lymphoma

D. Lymphoprolifertive Disease
Chronic lymphocytic leukemia

E. Renal Disease

F. Liver Disease
Biliary cirrhoiss
Laennec's cirrhosis
Viral hepatitis (acute and chronic)

G. Familial

H. Essential

*This table is not exhaustive, but is instead intended to represent some of the more common disease associations. Modified from Gorenic et al. (12)

The signs and symptoms of cryoglobulinemia are shown in Table II. Many of the clinical manifestations seem to result from low temperature-induced or increased local plasma protein concentration-induced cryoprotein aggregation, with consequent hyperviscosity of the blood. However, in situations in which cryoglobulinemia reflects the presence of circulating soluble immune

complexes, clinical manifestations are often due to inflammatory vascular injury mediated directly by the immune complexes.[5]

Table II. Incidence of Signs and Symptoms in Patients with Cryoglobulinemia*

Symptom	**Percent**
Cutaneous:	80
Vascular purpura	60
Distal necrosis	14
Urticaria	10
Livedo	10
Leg ulcers	5
Raynaud's phenomenon	50
Acrocyanosis	10
Arthralgia/arthritis	35
Nephritis	20
Neurologic	17
Hemorrhage	7
Symptom	**Percent**
Abdominal pain	2
Arterial thrombosis	1

*Modified from Brouet et al. (6) and Winfield (5)

Brouet et al.[6] proposed a classification of cryoglobulins in 1974 which continues to remain widely accepted.

Type I – these cryoglobulins consist of a monoclonal immunoglobulin. The most common diagnoses in patients with Type I cryoglobulinemia are multiple myeloma, Waldenström's macroglobulinemia, or other lymphoproliferative diseases.

Type II – these cryoglobulins are mixed cryoglobulins, composed of immunoglobulins of two classes, one of which is monoclonal. The monoclonal component always has rheumatoid factor activity (that is, reactivity against IgG); the second component is a polyclonal IgG which behaves as an antigen for the monoclonal rheumatoid factor. Type II cryoglobulins are characteristic

of the syndrome of "essential" mixed cryoglobulinemia, but occur in lymphoproliferative and autoimmune disorders as well.

Type III – these cryoglobulins are also mixed cryoglobulins, but both of the constituent immunoglobulins are polyclonal. Most Type III cryoglobulins contain a polyclonal IgM rheumatoid factor and polyclonal IgG. Type III cryoglobulins are the most common type encountered in clinical practice, and are associated with a variety of autoimmune and systemic connective tissue diseases, as well as with persistent infections.5

A variety of antibody activities in addition to anti-IgG activity (that is, rheumatoid factor activity) have been detected in cryoprecipitates. These antibody activities include anti-nuclear, anti-red blood cell, anti-complement, anti-cytomegalovirus, anti-hepatitis B, and a variety of anti-streptococcal activities. Similarly, a variety of antigens have been detected in addition to IgG itself, including nuclear antigens, renal tubular epithelial antigens, cytomegalovirus antigens, and hepatitis B antigens. Consequently, many cryoglobulins appear to represent immune complexes consisting of non-IgG antigen, specific antibody to the non-IgG antigen, and rheumatoid factor against this specific antibody. Complement components and fibronectin have also been detected in many Type II and Type III cryoprecipitates.

In two-thirds of patients with essential mixed cryoglobulinemia (Type II or Type III cryoglobulins with no known underlying etiology) there are associated liver abnormalities.7 Hepatitis C virus (HCV) now appears to cause the majority of these cases, with HCV RNA detected in the serum specimens of 90% of patients with essential mixed cryoglobulinemia and in none of patients with Type I cryoglobulinemia.8,9 Currently available methods of detecting HCV infection (anti-HCV antibody by enzyme-linked immunoassay) can detect approximately 50% of the HCV infected essential mixed cryoglobulinemia patients.7

Structurally, cryoglobulins are not significantly different from their noncryoprecipitating counterparts; cryoprecipitation is related more to electrostatic interactions and solubility than immunoglobulin structure.10 The molecular basis for the reversible cold precipitation of cryoglobulins is not fully understood.

For Type I cryoglobulins, it is thought that reduced solubility of the immunoglobulin is secondary to a temperature-dependent conformational change which promotes polymerization, and thus decreased solubility.

However, for the mixed cryoglobulins, that is Types II and III, it is thought that the cold-enhanced binding of the cryorheumatoid factor to polyclonal immunoglobulin in some way promotes insolubility.10,11

Treatment -Treatment of cryoglobulinemia is directed, ideally, at the underlying primary disease. In addition, treatment is also directed toward minimizing signs and symptoms of cryoglobulinemia itself.

Methods aimed at reducing the production or circulating concentration of cryoglobulins include drug therapy and plasmapheresis or plasma exchange (the replacement of a patient's plasma by normal donor plasma or a suitable substitute). In one long term study,12 a wide range of medications was employed for symptomatic treatment of mixed cryoglobulinemia, including nonsteroidal anti-inflammatory drugs, antihistamines, dextran, corticosteroids, and other immunosuppressive drugs. A number of clinical studies have demonstrated that plasmapheresis, plasma exchange, or cryofiltration (a variant of plasmapheresis in which macromolecules are removed by cooling the plasma to a selected temperature which is then filtered through a porous membrane) are particularly efficient at producing significant clinical improvement as well as reduced cryoglobulinemia.13-15 These effects include blood flow improvement, skin ulcer healing, reversal of impaired renal function, and disappearance of purpura and other abnormalities.16 Therapeutic plasma exchange procedures are normally carried out every other day with the aim of exchanging 1.0 to 1.5 times the plasma volume of the patient. In most cases, plasma exchange is employed as an initial, emergency treatment and can be combined with simultaneous cytotoxic or immunosuppressive therapy to prevent further production of the cryoglobulin. It should be noted that circulating levels of mixed and monoclonal IgM cryoglobulins are reduced more easily than those of IgG cryoproteins, most likely due to their removal from the intravascular pool with little equilibration with the extravascular pool.

The possibility that HCV infection is responsible for a majority of essential mixed cryoglobulinemia cases has led to therapeutic trials using interferon alpha to treat these patients. The early results of these trials appear promising.17,18

Laboratory Measurement of Cryoglobulins Patient blood samples submitted to the Clinical Immunology Laboratory for cryoglobulin analysis must be handled in a meticulous manner, because a significant number of cryoglobulins begin precipitating at temperatures only a few degrees below body temperature

(samples which have been allowed to cool below 32°C may give false negative cryoglobulin results, and consequently will not be tested). Blood samples for cryoglobulin should consist of 30 ml of blood drawn into three pre-warmed orange/green-top clot tubes. These samples should then immediately be transported to the Clinical Immunology Laboratory in 37°C sand or water. Warm tubes and transport containers filled with 37°C sand can be obtained from the Phlebotomy Division (362-1488). In the Clinical Immunology Laboratory, the specimen is allowed to clot at 37°C, and the serum is then refrigerated at 4°C; after 48 hours it is examined for the appearance of a cryoprecipitate (which may vary in appearance from a gelatinous mass to a fluffy white precipitate). If a cryoprecipitate forms, it is quantitated, and reported as micrograms per ml of serum.

All positive specimens are analyzed for specific immunoglobulin content by immunofixation electrophoresis. The technique of immunofixation electrophoresis, in which gel electrophoresis of the cryoprecipitate is followed by incubation with antisera specific for individual immunoglobulin isotypes, allows identification of the isotype and clonality of immunoglobulins that compose the cryoglobulin. In addition, all positive specimens are also analyzed for rheumatoid factor activity by an assay that measures the ability of the patient's serum to agglutinate IgG-coated latex particles.

This complete workup is used for the initial diagnosis and characterization of cryoglobulinemia. Treatment with plasmapheresis or interferon alpha can also be monitored by following the level of cryoglobulin. Alternatively, serum protein electrophoresis on samples collected and processed at 37°C can be used in patients with monoclonal cryoglobulins (Type I or Type II) to follow the level of the abnormal restricted peak which is reflective of their overall cryoglobulin level.

Interference with Other Laboratory Tests - If undetected, cryoglobulins can interfere with many laboratory tests by precipitating at room temperature and removing other proteins of clinical interest. In addition, cryoproteins can cause marked changes in automated CBC results due to precipitated protein aggregates, resulting in falsely elevated platelet and white blood cell counts,19 and can interfere with antibody screening and compatibility tests needed prior to RBC transfusion.20 In addition, levels of substances such as complement components and immunoglobulins may be falsely decreased due to cryoglobulin precipitation. If a patient with cryoglobulin is identified, the best way to obtain valid platelet and WBC counts, as well as levels of various serum proteins, is to keep the specimen at 37°C until analysis. Correct collection and

handling procedures for specific tests in question should be discussed with the appropriate laboratory supervisor or laboratory medicine resident.

Test Availability - The Clinical Immunology Laboratory performs cryoglobulin analysis daily with the initial results available two days after the receipt of the warm blood specimen. Results are reported out as negative, positive, or questionable normal (for samples which have an initial low concentration of precipitate which dissolves during the cold wash step of the cryoglobulin procedure). Samples found to be positive for the presence of cryoglobulin require an additional 1-2 days for quantitation and immunoglobulin and rheumatoid factor analysis.

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Management Experience: This includes a discussion of organizational behavior, organizational dynamics, negotiation, mediation, conflict resolution, strategic planning, quality improvement, capital budgeting, basic accounting, finance, human resource management, ethics, compliance, and health law.

Clinical Chemistry Pearls:

STATISTICS:

WESTGARD RULES:

1_{2s} = 1 observation ± 2s of mean (warning only)

1_{3s} = 1 observation ± 3s of mean (rejection)

R_{4s} = 1 observation +2s and 1 observation -2s of the mean (rejection)

2_{2s} = 2 consecutive observations + or - 2s of the mean (rejection)

41s = 4 consecutive + or - 1s observations (rejection)

10x = 10 consecutive observations on one side or the other of the mean (rejection)

*if a QC reading is on the line is it in or out?

IT IS LIKE TENNIS - IT IS IN!

SENSITIVITY

= POSITIVITY IN DISEASE EXPRESSED AS A %

= (TP/TP+FN) x 100

SPECIFICITY

= NEGATIVITY IN THE ABSENCE OF DISEASE

= EXPRESSED AS A % (TN/TN + FP) x 100

POSITIVE PREDICTIVE VALUE

= (TP/TP + FP) x 100

NEGATIVE PREDICTIVE VALUE

= (TN/TN + FN) x 100

EFFEICIENCY

= % OF PATIENTS CORRECTLY CLASSIFIED AS DISEASED OR NONDISEASED

= (TP + TN/TP + FP +FN + TN) x 100

STANDARD DEVIATION

= Ã·(xi - mean)^2 / n - 1

COEFFICIENT OF VARIATION

= (s/mean) x 100

T-TEST = (mean1 - mean2) / ÃS_1^2/N1 + S_2^2/N2

degrees of freedom = (n_1 - 1) + (n_2 - 1)

analyses the difference between means

F-TEST = is used to test for inequality of variances in the two population groups before the standard unpaired Student t-test can be applied.

CHI-SQUARE TEST = measures differences in proportion

ROC PLOT = A plot of the proportion of false positives vs true positives (this is done for specificity and sensitivity at various cutoff values to select the best cutoff value.

TONKS ALLOWABLE ERROR LIMIT FOR PRECISION = (1/4 NORMAL RANGE x 100) / NORMAL MEAN

NONPARAMETRIC OF NORMAL RANGE DISTRIBUTION = larger sample size, makes no assumption as to distribution and is greatly influenced by outliers.

GAUSSIAN DISTRIBUTION:
68.3% OF ALL VALUES ARE ± 1 standard deviation of the mean
95.5% OF ALL VALUES ARE ± 2 standard deviations of the mean
99.7% OF ALL VALUES ARE ± 3 standard deviations of the mean

OUTLIERS:
Throw out any observation for which $(y-y1) > 4S_e$
Throw out any value if the difference is greater than 1/3 of the range of all values
For a run statistical outliers are defined as values lying more than three SD from the mean

ANALYTICAL GOAL:
CV_a ² 1/2 $CV_{intraindividual}$

DETECTION LIMITS:

The detection limit of an immunoassay can be defined as the smallest concentration which can be statistically distinguished from zero. The mean ± 2 SD is often used to calculate this.

GENERAL CONCEPTS:

WATER:
Type 1 - used in test methods, highest purity, should be used immediately after production, Resistivity = 10 Mohm-cm, filtered, 0.2 µm and passed through an activated carbon filter

UNITS: don't forget to convert to mEq/L, you must first convert to liters, divide by the atomic weight of magnesium (for example), and **multiply by the valence**

BIOHAZARD:
In the 4 diamond system,

- numbers 0 to 4 qualitatively indicate degree of hazard (**4 being the highest hazard**)
- the top **red** diamond indicates flammability hazard,
- the left **blue** diamond indicates health hazard,
- the right **yellow** diamond indicates reactivity-stability hazard, and the bottom **white** diamond indicates special hazard information (such as radiation, biological hazard, etc.)

RADIATION:

Types:

α = helium nucleus, 2 proton and 2 neutron, mostly from heavy elements

β = negative electron emission

γ = high energy electromagnetic radiation (no plastic)

Half lives:

^{3}H	12.3 years
^{14}C	5730 years
^{32}P	14.3 days
^{35}S	87.1 days
^{51}Cr	27.8 days
^{57}Co	270 days
^{58}Co	71.3 days
^{60}Co	5.26 years
^{125}I	60 days
^{131}I	8.1 days

Units:

CONVENTIONAL

= curie (Ci)= 3.7×10^{10} DPS or 2.22×10^{12} DPM

SI = becquerel (Bq) = 1 DPS

1 curie = 3.7×10^{10} becquerels

Exposure = ionization produced by the radiation

CONVENTIONAL
= roentgen (R) = amount of radiation required to produce ions carrying one electrostatic unit of electric charge per cubic centimeter of dry air at STP.
SI = coulomb per kilogram (C/kg)

1 roentgen = 0.258 millicoulombs/kilogram

Radiation dose = amount of energy absorbed

CONVENTIONAL
= rad = 100 ergs absorbed per gram of matter
SI = gray (Gy) = one joule absorbed per kilogram
1 gray = 100 rad

GAMMA COUNTER = crystal scintillation detector (sodium iodide crystal). For ^{125}I the counting efficiency is about 70%.

LIQUID SCINTILLATION COUNTER = light (from scintillation fluid) is collected and converted to an electronic pulses β emitters such as ^{14}C and ^{3}H have unquenched counting efficiencies of 90% and 60% respectively.

COUNTING ERROR = Ãmean of counts

CENTRIFUGATION:

The relative centrifugal force = $1.118 \times 10^{-5} \times r \times n^2$
r = radius measured from the center of rotation to the bottom of the tube
n = speed in RPM

OSMOLALITY:

= osmotic coefficient **x** number of particles into which each molecule in the solution potentially dissociates **x** concentration in mol/kg H_2O or moles of all particles per kg of water

Calculated osmolality = 2[Na^+ in mmol/L] + [glucose in mg/L]/180 + [BUN in mg/L]/28 + 9 or

2[Na^+ in mmol/L] + 0.056 [glucose in mg/L] + 0.36 [BUN in mg/L] + 9

and the **Osmolal gap** is: measured - calculated
used to follow poisonings (e.g. glycol etc.), correlates well to toxicity and shows increases not detected by calculating osmolality.

When a solute is added to a solvent the:
1) osmotic pressure increases
2) vapor pressure decreases
3) boiling point increases
4) freezing point decreases

The **vapor pressure osmometer** introduces error because volitile substances such as ethanol, carbon dioxide, ethylene glycol, methanol etc. escape from the solution and increase the vapor pressure instead of lowering it.

COP or membrane osmometer measures the contribution of macromolecules to the osmolality. In the case of serum it is a measure of the contribution of serum proteins (normally small). It is more accurate than a serum protein measurement and is useful in guiding postoperative intravenous fluid therapy to prevent complications (eg. pulmonary edema). Standing values are higher than reclining because standing exerts a greater hydrostatic force on the vascular fluid forcing it out of circulation and into the interstitial fluid. This concentrates the serum proteins and increase COP.

Henderson Hasselbalch
HA = H+ + A-
BA = B+ + A-

dissociation constant for a weak acid = Ka = [H+] [A-] / [HA]

or [H+] = Ka x [HA] / [A-]

log [H+] = log Ka + log [HA] / [A-]

multiply by -1 to get pH

-log [H+] = -log Ka - log [HA] / [A-]

pH = pKa + log [A-] / [HA]
since [A-] is derived primarily from salt then

pH = pKa + log [salt] / [acid]
for blood; salt = bicarbonate (HCO_3-) and acid = CO_2 (dissolved CO_2 including undissociated carbonic acid) or **pH = pKa + log [HCO_3-] / [CO_2]**

pH = 6.103 + log (cHCO_3- / 0.0306 x pCO_2)

0.0306 is the solubility constant for CO2
6.103 is negative log of the combined dissociation constant for carbonic acid

Greatest buffering capacity is when pH = pKa

PHOTOMETRY:

Wavelength (nm)	**Region**	**Color**
<380	UV	not vis.
380-440	vis.	violet
440-500	vis.	blue
500-580	vis.	green
580-600	vis.	yellow
600-620	vis.	orange
620-750	vis.	red
750-2000	short IR	not vis.
1mm - 1m	microwave	not vis.

Beer's Law: A = abc

A = absorbance
a = molar absorptivity (constant)
b = length of light path (internal cell or sample length)
c = concentration (mol/L)

In toxicology or when the molecular weight of a substance is unknown people work with g/dL rather than mol/L. For b=1 and c=1g/dL (1%) A can be written as $A^{1\%}_{1cm}$ which is called the **absorption coefficient**, $E^{1\%}_{1cm}$ is an older obsolete symbol called the **extinction coefficient**.

Spectral purity is described in terms of its spectral **bandwidth**. It is defined as the width in nanometers of the spectral transmittance curve at a point equal to one half the peak transmitance. Measured using interference filters or a mercury lamp. **Wide-bandpass filters** have spectral bandwidths of about 50 nm. Narrow spectral bandwidths (5-15 nm) are reffered to as **interference filters**.

A **prism** separates white light into a continuous spectrum by refraction, that is, shorter wavelengths are bent, or refracted, more than longer wavelengths as they pass through the prism.

The **natural bandwidth** of an absorbing substance is defined as the bandwidth of the spectral absorbance curve at a point equal to one half of the maximum absorbance.

Wavelength calibration: holmium oxide, didymium, mercury lamp or benzene vapor

Photometric Accuracy: potassium dichromate or NBS neutral glass filters

Stray light: The major effect of stray light is an absorbance error especially in the upper end of the absorbance range of the instrument.

Flame Photometry:

Atoms of many metallic elements, when given sufficient energy such as that supplied by a hot flame, will emit this energy (about 1-5% of the atoms) at wavelengths characteristic for the element. A specific amount or quantum of

thermal energy is absorbed by an orbital electron, which, being unstable, in this excited state release their excess energy as photons of a particular wavelength as they return to their ground state. If the energy is dissipated as light, the light may consist of different wavelengths called line spectra which are characteristic for each element. Sodium for example emits mainly at 589 nm with others of much lesser intensity.

For sodium and potassium measurements a temperature of about 2,000 ¡C is adequate (propane and air).
Lithium = red, sodium = yellow, potassium = violet, rubidium = red, and magnesium = blue

Addition of internal standard allows compensation for small variations in atomization rates, flame stability, and solution viscosity.

The potassium signal is enhanced by the sodium concentration in the sample (mutual excitation) and may be a problem when direct reading method is used.

Atomic Absorption:

Principle - Sample is not appreciably excited in flame but only dissociated from its chemical bonds and put in the ground state where it can absorb radiation at a narrow bandwidth corresponding to its own line spectrum. For example a hollow cathode lamp with the cathode made of sodium, sodium light ~589 nm is emitted by the lamp. When the light from the lamp enters the flame some of it is absorbed by the ground state atoms in the flame resulting in a net decrease in the intensity of the beam. This process is called atomic absorption.

Flame:
total consumption burner = Good because; flame is more concentrated and can be made hotter causing molecular dissociation that may be desirable (eg. calcium phosphate complexes). Bad because its acoustically noisy and because large droplets enter the flame light is scattered and the signal is noisy

premix burner (laminar flow burner) = Good because; large droplets go to waste while a fine mist enters the flame giving less noise, also the pathlength through the flame is longer giving greater absorption and increased sensitivity. Bad because flame isn't as hot and can't cause dissociation of some metal

complexes (eg. calcium phosphate complexes).

Flameless:

The sample is placed in a depression in a carbon rod (can also use tantalum or platinum) in an enclosed chamber. Increase temperature to atomize. The atomized element then absorbs energy from from the corresponding hollow cathode lamp. Good because its more sensitive (can use small samples and detect trace elements). **Zeeman effect** = in an intense magnetic field, the energy levels that define the wavelength of the emitted radiation are slightly shifted. Therefore if monitoring constantly at the analytes wavelength and pulsing the magnetic field you shift the analyte slightly and get a very accurate background reading.

Interference:

chemical - e.g. calcium phosphate complexes, use strontium or lanthanum to displace calcium

ionization reference - atoms in the flame become excited, instead of being only dissociated, overcome by adding an excess of a more easily ionized substance that will absorb the flame energy so that the substance of interest will not become excited, or turn down temperature

Luminescence is the emission of light or radiant energy when an electron returns from an excited or higher energy state to a lower energy level. Luminescence includes fluorescence, phosphorescence, and chemiluminescence

Fluorescence occurs when a molecule absorbs light at one wavelength and re-emits light at a longer wavelength.

Phosphorescence shows a larger shift in emitted light wavelength and longer decay time than does fluorescence.

In **chemiluminescence and bioluminescence** the excitation event is caused by a chemical or electrochemical reaction and not by photolumination. Sensitivity is attamole (10^{-18}) to zeptomole (10^{-21}) detection limits.

Bioluminescence is a special form of chemiluminescence in which an enzyme or a photoprotein (eg. luciferase or aquorin) increases the efficiency of the luminescent reaction.

Electrochemiluminescence differs from chemiluminescence and bioluminescence in that the reactive species that produce the chemiluminescence reaction are electrochemically generated from stable precursors at the surface of the electrode (limits of detection = 200 fmol/L and a dynamic range extending over 6 orders of magnitude.
The time required for the emitted light to reach 1/e of its initial intensity, where e is the Naperian base 2.303, is called the average lifetime of the excited state of the molecule, or the **fluorescence decay time**.

Time resolved fluorometers exploit the time delay between absorption of quanta of energy and fluorescence. The advantage is that they eliminate background light scattering due to Rayleigh and Raman signals and short lived fluorescence background increasing signal-to-noise and detection sensitivity.
2 categories –
1) **pulse fluorometry**, in which the sample is illuminated with an intense brief pulse of light and the intensity of the resulting fluorescence emission is measured as a function of time with a fast detector system.
2) **phase fluorometry**, in which a continuous-wave laser illuminates the sample and the fluorescence emission response is monitored for impulse and frequency response.

Fluorescence Polarization:

Light is composed of electrical and magnetic waves at right angles to each other. Light waves produced by standard excitation sources have their electrical vectors oriented randomly. Light waves passed through certain crystalline materials (polarizers), have their electrical vectors oriented in a single plane are said to be plane-polarized. Fluorophors absorb light most efficiently in the plane of their electronic energy levels. If their rotational relaxation (Brownian movement) is slower than their fluorescence decay time, as is the case for large fluorescence-labeled molecules, the emitted fluorescence light will be polarized. Since small molecules have rotational relaxation times that are much shorter than their fluorescence decay time their emitted fluorescence light is depolarized unless it is attached to a macromolecule or put in a viscous solution.

$$P = (I_V - I_h) / (I_V + I_h)$$

I_V= emitted fluorescence in the vertical plane

I_h= emitted fluorescence in the horizontal plane

Excitation source:

300-550nm absorption spectra, xenon arc lamp, laser, quartz halogen cycle lamp, mercury arc lamp

Limitations:

- concentration effects (inner filter, quenching)
- background effects (Rayleigh and Raman scattering)
- solvent effects (interfering fluorescence and quenching)
- sample effects (adsorption, scattering, quenching)
- temperature effects
- photodecomposition (bleaching) of the sample

Front surface minimizes inner filter effects and provides the greatest linearity but is susceptable to background scatter.

Rayleigh scattering expression describes the relationship of scattered to radiant energy as a function of particle diameter and wavelength of incident light.

Isosbestic point = not isoelectric point! it is the wavelength at which absorbances are identical for two interconvertible substances.

Allen correction for background absorbance = Ax = $A_{\lambda x}$ - 1/2 ($A_{\lambda 1}$ + $A_{\lambda 2}$)

ELECTROCHEMISTRY:

Potentiometry = the measurement of the electrical potential difference between 2 electrodes in an electrochemical cell.

ion activity - free or unbound ion, based on the potential of the unknown sample compared to that of several standards of known activity (direct potentiometry).
ion concentration - total ion (dilute and dissociate or indirect potentiometry)
Electrodes include , inert metals (gold and platinum, platinum-hydrogen or quinhydrone), silver, calomel (mercury chloride), and ion selective electrodes

ISE:

reference = silver electrode
test = calomel electrode (diffusible cation activity higher here)

Nernst equation = membrane potential is directly proportional to the logarithm of the activity of the diffusible ion in the test solution.

Voltammetry: Technique used to study solution composition based on the current-potential relationships obtained when the potential of an electrochemical cell is varied (usually under the control of a three-electrode *potentiostat*). In this system the polarizable working electrode potential is measured potentiometrically (i.e. at zero current) between the working and reference electrodes, and the cell current is measured between the working and counter (or auxillary) electrodes. This eliminates errors caused by the current flow through the resistive solution (called IR drop).
Because of the wide variety of excitation (potential-time) waveforms that can be used, there is a broad repertoire of voltammetric methods, including linear potential sweep (e.g. classical polarography and cyclic voltammetry), potential step (e.g. normal and differential pulse, and square-wave voltammetry), anodic and cathodic stripping voltammetry, and phase-sensitive AC voltammetry.

Polarography is the term applied the special case where the working electrode is a dropping mercury electrode (renews electrode surface, reducing possibility of electrode fouling). Polarography is based on the dual measurement of the current flowing through an electrochemical cell and the electrical potential between the 2 electrodes when the potential is gradually increased at a constant rate using an external voltage source.

Anodic stripping volammetry uses a mercury coated graphite rod. When a negative potential is applied to the electrode the trace metal ions of the sample are reduced and plate the electrode (` 1-30 min, concentration here). A voltammogram is recorded with the plated electrode as the anode and a nonpolarizable cathode. The metals are stripped off the anode by oxidation of the respective ions. The order in which they are stripped off is a function of the metal's unique redox potential. The current flow during the stripping of a given metal is a function of the amount of metal present (very sensitive method).

Potentiometric stripping analysis is a modification of the above. After the first spet the electrode is left undisturbed and the cell potential is measured as a function of time. The electrode reactions proceed in the reverse direction because of the presence of oxidizing species in the solution (oxygen or Hg^{2+}). During this process the potential remains constant and the length of the plateau on the potential/time curve is proportional to the concentration of the first metal (chronopotentiometry). When the next metal starts to strip a jump in potential is observed and the process is repeated.

Amperometry is based on measurement of the current flowing through an electrochemical cell when a constant potential is applied to the electrodes (e.g. pO_2 electrode). Sample is acidified and Cl^- is titrated with Ag^+. Ag^+ concentration remains low due to precipitation of Cl^- as AgCl. At the end point Ag^+ appears in excess and this can be detected either potentiometrically or amperometrically.

Coulometry is the technique used to measure the amount of electricity passing between 2 electrodes in an electrochemical cell. The amount of electricity is directly proportional to the amount of substance produced or consumed by the redox process at the electrodes. This is called Faraday's law. An example of this is listed above for Cl^-.

Biosensor is a device or system in which an immobilized biological/biochemical component interacts with the analyte to produce, via an appropriate transducer, a signal proportional to the quantity or activity of analyte.

SEPARATION METHODS:

Electrophoresis:

Zone electrophoresis - describes the migration of charged macromolecules in a porus supporting medium (e.g. agarose, cellulose acetate, cellulose paper).

Isotachophoresis is a technique in which sample components ultimately separate into adjacent zones that all migrate at the same rate. Sample is placed in a capillary between a leading electrolyte solution that contains ions that are faster than any in the sample and a trailing solution containing ions that are slower that any in the sample (no background electrolyte - buffer is used). Applications include small anions and cations, organic and amino acids, peptides, nucleotides, nucleosides and proteins.

Buffer effects: carries the applied current and determines the pH, increasing concentration of buffer decreases molecule motion and sharpens resolution but increases denaturation of heat labile protein.

An electrophoretic support medium in contact with water takes on a negative charge because of adsorption of hydroxyl ions. Positive ions in the solution cluster about the fixed negative charge sites, forming an ionic cloud of mostly positive ions. The potential that exists between the fixed ions and the associated cloud of ions is termed the **zeta or electrokinetic potential (ζ)**. **Electroendosmosis** is the movement of solvent and its solutes relative to the fixed support. Macromolecules in solution that move in the opposite direction oppose this flow of hydrated positive ions. If the molecules are insufficiently charged they remain immobile or may be swept toward the opposite pole (e.g. γ-globulins in cellulose acetate). These effects are minimal in media where surface charges are minimal (starch or PAGE), and can be reduced by removing or modifying sulfate or carboxylic acid groups or adding sucrose or sorbitol.

Stacking or spacer gel serves to concentrate protein components at the border or starting zone (all proteins migrate through the large-pore gel(s) and stack up. Disc (= discontinuities in the electrophoretic matrix) electrophoresis overcomes broad protein bands.

Isoelectric focusing (IEF) separates compounds by virtue of migration in a medium possessing a stable pH gradient, with pH varying in the direction of the migration. The pH gradient is created by the use of amphoteric polyaminocarboxylic acids (or carrier **ampholytes**, MW = 300-1000)

2 D Electrophoresis: **O'Farrell** uses PAGIEF tubes in the first direction and SDSPAGE (slab) in the second direction.

Capillary electrophoresis: advantages include increased heat dissipation, reduced sample volume, reduced zone broadening and easier process automation.

CHROMATOGRAPHY:

Surface Adsorption - basis is the electrostatic, hydrogen-bonding, and dispersive interactions between a molecule and a solid support or adsorbent.

Partition chromatography - based on differences in the solubility of individual solute molecules in two immiscible liquids that are in contact with one another (or LLC).

Partition or distribution coefficient - the ratio of the solubility of the solute in the extracting solvent (stationary phase) to its solubility in the original solvent (mobile phase).

Resolution = difference in retention times Ö 1/2 the sum of the base peaks

Efficiency = number of **theoretical plates** (N). A plate is the length of a column needed to allow one equilibration of the solute to occur between the stationary and the mobile phases. $N=5.5\ [V_r(A)/1/2\ W]$ where 1/2W is the peak width at half height, and V_r is the retention time of peak A. **Height equivalent theoretical plate** or H = N/L where L = column length.

Rf - distance of spot migration Ö distance of mobile phase migration

Capacity Factor - ratio between the concentration of the solute molecules in the stationary phase relative to the mobile phase (same as R_f in planar work).

Selectivity Factor - the measure of the relative separation between the peak or band centers of 2 solutes.

IMMUNOLOGY:

Hapten = is a chemically defined determinant that when conjugated to an immunogenic carrier stimulates the sythesis of antibody specific for the hapten. It is capable of binding antibody but cannot by itself stimulate an immune response.

Affinity = refers to a single antibody-combining site and its corresponding epitope on the antigen.

Avidity = refers to the overall strength of binding of antibody and antigen and includes the sum of the binding affinities of all the individual combining sites on the antibody.

Ouchterlony technique = double immunodiffusion in 2 directions

Rocket immunoelectrophoresis = a single concentration gradient with an applied voltage used to drive the antigen from the application producing a unidirectional migration of the antigen and results in increased sensitivity.

Counter immunoelectrophoresis = a voltage is applied across a gel with 2 parallel lines of wells containing Ab in one set and Ag in the other. Antigen moves towards the anode and antibody towards the cathode.

Nephlometry - more sensitive than turbidimetry uses the 90¡ angle measurements

Heterogenous immunochemical assay = separation of free label and bound label vs **homogenous** which does not require separation.
Labelled antibody assays = **IRMA** or immunoradiometric assay

Competitive immunoassay = labelled and unlabelled antigen compete for binding to the antibody (must have equal avidity)

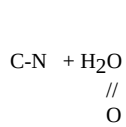
Noncompetitive immunoassay = a capture antibody is passively adsorbed or covalently bound to the surface of a solid phase, then antigen from the sample is allowed to react with the solid phase antibody and after washing a labelled antibody reacts with the bound antigen.

ELISA = enzyme-linked immunosorbent assay

EMIT = antibody is added together with substrate to the patients sample, binding occurs and an enzyme conjugate of the analyte is added which also binds the excess analyte antibody. Binding of the analyte antibody with the enzyme-analyte conjugate affects enzyme activity by physically blocking access of the substrate to the active site of the enzyme or by changing the conformation of the enzyme molecule and thus altering activity. Assay enzyme activity.

FPIA = homogeneous assay

PROTEINS AND NITROGEN METABOLISM:

Peptide bond - 

 sheet - polypeptide chains of the extended coils that are bound together laterally by disulfide covalent bonds and hydrogen bonds (same way is parallel and opposite direction is antiparallel.

 helix - rodlike structure of tightly coiled polypeptide main chain forms the inner part of the rod, and the side chains extend outward in a helical array. The α helix
is stabilzed by hydrogen bonds between the NH and CO groups of the main chain.

IgM = MW of 900,000 and exists as a pentamer in the serum but is a monomeric membrane receptor. Accounts for 5-10% of the total circulating immunoglobulins and is the only immunoglobulin that a neonate synthesizes. It activates complement and is not transported across the placenta.

IgG = 70-75% if the total immunoglobulins. 65% is extravascular, MW is 160,000 and has 4 subclasses. 1 and 3 activate killer monocytes and 1 is the principal IgG to cross the placenta by an active transport process dependent on Fc binding.

IgA = 10-15% of the immunoglobulins, MW = 160,000 (exact role not clear). Secretory form has a MW of 380,000 (dimer +) synthesized by plasma cells can activate complement and is present in milk and colostrum probably to protect neonates from intestinal infections.

IgD = less than 1%, monomeric, MW = 184,000 and is a surface receptor for antigen in B-lymphocytes, role unknown

IgE = rapidly and firmly bound to mast cells, MW = 188,000. When antigen crosslinks 2 of the attached IgE molecules, the mast cell is stimulated to release histamine and other vasoactive amines (allergic reactions).

Paraproteins (monoclonal immunoglobulins)

A single clone of plasma cells (plasmacytes are active in antibody formation and found in R.E. and bone marrow) produces immunoglobulin molecules with identical structures. If the clone is permitted to multiply, the concentration of its particulr protein in the patients serum becomes so great that on electrophoresis it often produces a narrow, sharply discrete spike. These monoclonal immunoglobulins may be polymers or polymers of monomers or fragments (if fragments they are usually light chains called **Bence Jones** proteins) or rarely heavy chains. 60% are due to multiple myeloma or to a single plasmacytoma and 15% are due to overproduction of β-lymphocytes (eg lymphomas, leukemias, **Waldenstršm's** macroglobulinemia - IgM) and about 25% are benign.

Methods - electrophoresis - albumin, $\alpha 1$, $\alpha 2$, β, ($\beta 2$, C3 complement- if fresh) and γ

- decreased albumin and γ with increased $\alpha 2$= nephrotic syndrome or proteinuria
- increase in $\alpha 1$, $\alpha 2$ suggests an acute phase reaction
- increased $\beta 1$ suggests iron deficiency anemia (transferrin increase) of high levels of estrogen
- fusion or bridging of β and γ suggests increased IgA as in cirrhosis or rheumatoid arthritis
- increased γ suggests a polyclonal γ globulin increase associated with an immune reaction, liver disease or disseminated neoplasms
- absence of γ suggests immune deficiency

Albumin-Biuret Reaction (about 6-8 g/dL):
When a solution of protein is treated with Cu(II) ions in a moderately alkaline medium, a colored chelate is formed between the Cu(II) ion and the carbonyl oxygen and amide (=NH) atoms of the peptide bond.

Bromcresol Purple - yellow BCP dye turns green with albumin, supposed to be very specific

AAT - acute phase and has antiprotease activity, although relatively inactive towards trypsin it is responsible for 90% of serum antitrypsin activity, main function is to neutralize lysosomal elastase released on phagocytosis of particles by polymorphonuclear leukocytes.

AAG - (alpha1-acid glycoprotein or orosomucoid), acute phase, low pI 2.7-3.5, associated with inflammation and functions in coagulation and hemagglutination, made in liver.

AFP - synthesized in the fetal liver, function unknown, used for screening of neural tube defects and as a tumor marker.

Haptoglobin - $\alpha 2$ region, made in liver, MW >80,000, binds hemoglobin therefore conserves iron, sports can lower

Alpha2-Macroglobulin - MW= 625,000-800,000, inhibits proteases, but functions poorly understood, may be important in immunological and inflammatory processes, not an APR

Ceruloplasmin - fast $\alpha 2$, late APR, major copper-containing protein and is reduced in Wilson's disease, provides a first line defence vs copper toxicity, antioxidant and APR.

Transferrin - or siderophilin, β migration, main iron transport protein, negative APR, useful in the diagnosis of anemia.

Beta2-microglobulin - test of renal function (MW = 11,800), increased in CSF in leukemia and lymphoma with CNS involvement and increased in salivary glands in Sjšgren's syndrome

CRP - MW 115,000 to 140,000 slowγ to the midβ, APR, MI, RA and Crohn's etc.

Amyloid - amyloidosis, AL, AA, Senile and Familial

Ferritin - estimates bone marrow iron stores

Urine Protein - (1-14 mg/dL)

Tamm-Horsfall protein or uromucoid is a constituent of urinary casts and probably secreted by the distal tubules.

Transudates - (change in membrane pereability) protein
< 3 g/dL

Exudates - (malignancy or infection) protein > 3 g/dL

Nitrogen Balance -

Urea - CON_2H_4 is the major nitrogen-containing metabolic product of protein catabolism in humans, accounting for more than 75% of the nonprotein nitrogen eventually excreted. More than 90% of urea is excreted through the kidneys, it is neither actively reabsorbed nor secreted by the tubules but 40-70% moves out of the renal tubule (diffusion) and into the interstitium, ultimately to re-enter plasma.

Measurements - indirect, urease-Berthelots reaction, and electrochemical detection of ammonium, direct - Fearon reaction (7-18 mg/dL)

Purines = A,G (2 rings)
Pyrimidines = C,T and (U), (single ring)

CREATININE: Creatine is synthesized in the kidneys, liver and pancreas. It is transported to the brain and muscle and converted to phosphocreatine (important in muscle contraction). Between 1-2% of muscle creatine is converted (spontaneously to its anhydride) to creatinine.
Amount is affected by age, sex and lean body mass. However excretion generally reflects production and is relatively constant within individual (±10-15%)
A small amount is reabsorbed by the tubules and a small amount is due to tubular secretion (~7%) becomes less useful when patient loses renal function (increased tubular secretion)

Jaffe reaction = picrate ion in alkaline medium (red orange)
interferences = glucose, ascorbic acid, guanidine, acetone, cephalosporins, acetoacetate, and pyruvate (increase), kinetic measurement is still subject to α ketoacids and bilirubin (negative), and enzyme measurement (uses ammonia) is subject to endogenous ammonia, creatine and environmental ammonia.
Definitive method = isotope dilution mass spectrometry
Reference method = isocratic HPLC with UV detection (234 nm)

Clearance:
ml of plasma cleared / min / standard surface area = (Ucr **x** V / Pcr) **x** 1.73 / A

Ucr = urine creatinine in same units as Pcr - plasma creatinine
(preferably mg/dl)
A = body surface (square meters)

1.73 factor normalizing body surface area (actually 1.73 m^2)
V = volume of urine flow in ml/min

Estimate from tables has problems from obesity (falsely elevated) and random error

URIC ACID: major catabolic product of purine nucleosides, adenosine and guanosine (~400 mg/day synthesized and 300 mg/day dietary) mostly (75%) excreted in urine (filtered, reabsorbed, secreted reabsorbed, net ~6-12% loss) reutilization of free bases via **HGPRT** (hypoxanthine-guanine phosphoribosyl transferase-major) and APRT **salvage pathways** (adenine phosphoribosyl transferase-minor)
pKa ~5.7 and acid form is less soluble, stones are radiolucent
Allopurinol - inhibits xanthine oxidase thereby suppressing purine synthesis and degradation of hypoxanthine to uric acid.
Lesch-Nyhan syndrome - complete deficiency of HGPRT, the major enzyme of the purine salvage pathway (mutilation)

Methods: Phosphotungstate- interferences by glucose, ascorbic acid, glutathione, ergothionine, cysteine, acetaminophen, ASA, genistic acid (an ASA metabolite), caffiene, theobromine, theophilline.
Uricase - more specific but guanine xanthine and a few other structural analogues interfere (most common method), urate to allantoin (decrease in urate absorbance ~290 nm.

BLOOD GASES AND ELECTROLYTES:

Sodium - hypo - obvious ones and deficiency of mineralocorticoids, metabolic acidosis, renal tubular acidosis, nephrotic syndrome, cirrhosis, SIADH, retention of water (dilutional), psuedo - lipemia, hypernatriuria
hyper - sweating, polyuria etc., salt intake (-water), increase in mineralocorticoids (Cushing's syndrome), insulin treatment of diabetes (glucose decrease causes Na+ into cells and decreased plasma osmo. causes contraction of extracellular fluid vol., hyponatriuria
(136-145 mmol/L, CSF = 136-150 mmol/L, sweat = 10-40 mmol/L, CF > 70 mmol/L)
Potassium - hypo -aldosterone reabsorbs Na+ and excretes K+ (exchange) and decreases reabsorbtion, alkalosis, vomitting, diarrhea, renal tubular acidosis, Cushing's or Bartter's syndromes, diuretics (thiazides, loop diuretics, carbonic anhydrase inhibitors, antibiotics, cirrhosis, Conn's syndrome and theophylline
hyper - I.V, shock, dehydration, diabetic ketoacidosis, severe burns, violent muscular activity, renal failure due to shock or renal tubular acidosis, acidosis, duretics (spironolactone, amiloride etc.) adrenocortical insufficiency, thrombocytosis and leukocytosis

Hemolysis a BIG problem, direct ISE best for analysis because it measures activity not concentration and isn't affected by lipemia etc.
In RBC its ~105 mmol/L in tissue cells ~150 mmol/L and in serum its 3.5 - 5.1 mmol/L, CSF ~ 70% of serum values

Chloride - serum = hypo - chronic pyelonephritis, salt losing nephritis, Addisonian crisis, metabolic acidosis (diabetic ketoacidosis and renal failure), metabolic alkalosis, aldosteronism, bromide intoxication, volume expansion
hyper - dehydration, renal tubular acidosis, acute renal failure, salicylate intoxication, diabetes insipidus, adrenocortical hyperfunction, metabolic acidosis associated with prolonged diarrhea and loss of bicarbonate (stool measurement is important)
98-107 mmol/L, RBC = 45-54 mmol/L

Coulometric-amperometric determination of Cl- depends on the generation of Ag+ from a silver electrode at a constant rate and on the reaction of Ag+ with Cl- in the sample to form insoluble AgCl. Once all the Cl- is titrated excess Ag+ in the mixture triggers shutdown of the Ag+ generation system. This is timed and the time interval is proportional to the amount of Cl- in the

sample. Use appropriate calibrators for sweat and interferences include other halide ions, sulfhyryl groups, CN-, SCN- and heavy metals

ISE is also popular, direct on Dupont and Ectachem, indirect on Abbott and Hitachi, indirect method is not accepted by the Cystic Fibrosis Foundation.

Bicarbonate or Total CO_2 - Bicarbonate ions make up all but **~2 mmol/L** of the total carbon dioxide of plasma: about 1 mmol/L of the bicarbonate exists as undissociated sodium bicarbonate. Air has much less CO2 than plasma, therefore can see a decrease in CO_2 of ~ 6mmol/L in an hour. Acidification of sample releases CO_2.

BLOOD GASES AND pH

Boyle's Law = the volume of an ideal gas at a constant temperature varies inversely with the pressure exerted to contain it
Charle's (Gay-Lussac's) Law = The volume of an ideal gas at a constant pressure varies directly with its absolute temperature.
Avagadro's Law = equal volumes of different ideal gases at the same temperature and pressure contain the same number of molecules
Dalton's Law = the total pressure exerted by a mixture of ideal gases is the sum of the partial pressures of each of the gases in the mixture
Henry's Law = the amount of a sparingly soluble gas dissolved in a liquid is proportional to the partial pressure of the gas over the liquid
General gas equation (Nerst) = PV = nRT

Henderson Hasselbalch equation for normal plasma at 37¡C:

pH = 6.103 + log ($cHCO_3$- / 0.0306 x pCO_2)
0.0306 is the solubility constant for CO2
6.103 is negative log of the combined dissociation constant for carbonic acid

Oxygen is reversibly bound to Fe(II) in hemoglobin (O_2Hb) and Hb exists as a tetramer. Oxygen dissociation curve is sO2 plotted against pO2 and is affected by 5 factors

1) pH > 7.4 (base) shift to the left
2) pH < 7.4 (acid) shift to the right
(note: the effect of pH to shift the curve is called the Bohr effect)
3) Temp > 37¡C shift to the right
4) Temp < 37¡C shift to the left

5) pCO_2 > 40 mm Hg shift to the left
6) pCO_2 < 40 mm Hg shift to the right

7) DPG(E) > normal shift to the right
8) DPG(E) < normal shift to the left
and type of Hb (obviously)
A shift to the right indicates a decrease in Hb affinity for O_2 while a shift to the left indicates an increased affinity of Hb for O_2

DPG(E) = 2,3-diphosphoglycerate in erythrocytes, its the predominant glycolytic intermediate of RBC's and stabilizes the deoxygenated configuration of Hb

Hill plot straightens sigmoidal curve

$pO_2(0.5)$ or P_{50} is the pO_2 at which hemoglobin of the blood is half saturated with O_2

CO-oximeter measures 4 hemoglobins simultaneously at four wavelengths and oxygen saturation can be calculated as follows:

%O2 sat'd = $[HbO_2] / [HbO_2] + [Hb_{red}] + [HbCO] + [Hb_{met}]$

The pH of freshly drawn blood decreases at a rate of 0.06 pH units per hour @ 37¡C but only 0.006 pH units/hr @ 4¡C.

blood pH = 7.40 ± 0.04 or 4 x 10^{-8} mol/L = 40 nmol/L assuming the activity of H^+ is 1

Base excess is the concentration of titratable base (calculated by a nomogram or by the Van Slyke equation)

Tonometry is the process of exposing a liquid to an ambient gas phase in such a way that each gas in the gaseous phase partitions to an equilibrium between the liquid and gas phases.

If a solution on one side of a membrane contains ions that cannot freely move through the membrane (e.g. proteins), distribution of the diffusible ions at the steady state is unequal; however, ***Gibbs-Donnan Equilibrium*** - *the product of the concentrations of diffusible ions in one compartment is equal to the product of diffusible ions in the other compartment*.

Plasma buffers, a) bicarbonate, b) plasma proteins (95% of non-bicarbonate buffers), and c) phosphate (5% of non-bicarbonate buffers)
In RBC hemoglobin makes up ~80% of the non-bicarbonate buffers with 2,3-diphosphoglycerate making up the remainder

Isohydric and Chloride Shift

Isohydric - (hydrogen ion remains constant) CO_2 moves from tissue to plasma and into RBC where it is acted on by carbonic anhydrase to give H^+ and HCO_3^-. The H^+ displaces O_2 from HbO_2 and O_2 moves out of the RBC.

Chloride - as bicarbonate moves out of RBC (don't ask why) Cl- moves in to provide electrochemical balance

Na^+-H^+ exchange in the tubules is energy dependent and the maximum urine acidity is ~pH 4.4 (potassium will compete)

Ammonia - at normal blood pH the ratio of NH_3 to NH_4^+ is about 1 to 100.

~60% of the H+ associated with nonvolitile acids is excreted as NH_4^+.

Potassium depletion increases ammonia genesis and K+ overload decreases it.

Bicarbonate reclamation (90% in proximal tubule) is actually CO_2 diffusion and not bicarbonate reabsorption and is caused by acidified urine.

METABOLIC ACIDOSIS

Cause - production of organic acids (diabetes, lactic acidosis, salicylate poisoning)

- reduced secretion of acids (renal failure, RTA)
- excessive bicarbonate loss (carbonic anhydrase inhibitors)

characterized by a high anion gap
for pH take bicarbonate and add 15 to get
(e.g. 8 + 15 = pH of 7.23)

for **pCO2 ± 2 take 1.5 (HCO_3^-) + 8 (e.g. 1.5(8) + 8 = 20)**

*** this is important clinically because it tells the clinician what it should be in the absence of compensation**

Compensation

- respiratory hyperventilation (Kussmaul respiration) to decrease CO_2
- renal, increased acid secretion and HCO_3 retention (**note** - a fully compensated metabolic acidosis may have a normal pH but still have a bicarb deficit)

METABOLIC ALKALOSIS

Cause - excess alkali
(bicarb, antacids, citrate from transfused blood)
- vomiting or intestinal obstruction
- potassium depletion
(Cushing's, licorice or low K+ intake)
- renal bicarb retention
(aldosterone stimulation due to hypovolemia)
- diuretics
- laxatives

Compensation
- respiratory hypoventilation

pCO2 ± 2 take 0.9 (HCO_3^-) + 9 (e.g. 0.9(8) + 9 = 16) * this is important clinically because it tells the clinician what it should be in the absence of compensation

- renal, decreased Na+ - H+ exchange decreased formation of ammonia and decreased reclamation of bicarbonate

Note - in potassium depletion urine pH may be low due to Na^+ - H^+ (paradoxical aciduria)

RESPIRATORY ACIDOSIS

Causes - respiratory center depression (drugs, trauma, meningitis, tumors etc.)

- disturbed respiratory apparatus (COPD – chronic obstructive pulmonary disease, adult respiratory distress syndrome etc.)

- other (extreme obesity - pickwickian syndrome, sleep apnea etc.)

Compensation

- respiratory - increase rate and depth (if possible)

pCO2 ± 2 take 1.5 (HCO_3^-) + 8 (e.g. 1.5(8) + 8 = 20) * this is important clinically because it tells the clinician what it should be in the absence of compensation

- renal (main)- same as metabolic acidosis (2-3 days)

Note - a so-called fully compensated chronic respiratory acidosis in which CO2 is high but pH is normal is actually not the result of a true compensation but is generally the result of a superimposed metabolic alkalosis (e.g. diuretics)

K^+ may be elevated due to H^+ movement into cells

RESPIRATORY ALKALOSIS

Causes - stimulation of respiratory center (fear, drugs, trauma, altitudes, anemia, asthma, congestive heart failure, pneumonia, ventilator)

Compensation

- first tissue and RBC buffers provide H^+ ions to consume HCO_3 then renal compensation (main) as for metabolic alkalosis

decreased K^+, increased ketone bodies if prolonged and phosphate decreased

ANION GAP (12-20 mmol/L)

$$= Na^+ - (Cl^- + HCO_3^-)$$

can include sodium and potassium

increased in metabolic acidosis, respiratory or metabolic alkalosis, apparent - hypokalemia, hypocalcemia etc.,
decreased hypoalbuminemia, hypergammablobulinemia, apparent - hypermagnesemia, hyperkalemia, or lithium toxicity

THERAPEUTIC DRUG MONITORING:

Volume of distribution = Dose / ÆC

First Order Kinetics - removal of the substance is inversly related to the concentration of the substance (renal and hepatic clearance typically exhibit) = $C = C_o * e^{-K_d t}$, C_o = maximun (at time 0), K_d = rate constant for clearance and t = time since administration of the drug

to determine the half life = 0.693/Kd, Kd = clearance constant

time between doses = 1/Kd x ln (Cnew peak/Cnew trough)

Zero Order Kinetics - usually occurs when the clearance is maxed out (constant) then we can no longer predict drug levels based on dose e.g. = phenytoin or dilantin but salicylate, ethanol, and occasionally theophilline will show zero order kinetics.

Changing drug levels:

For drugs administered at intervals equal to or less than the half life, when clearance is first order the assumption that the single observed concentration represents an average value not far from correct (e.g. doubling the dose should double the concentration)

For drugs administered at intervals greater than the half life:

Fractional clearance = peak - trough / peak, by multiplying the trough by this clearance value it is possible to predict the number of intervals required to reach the desired trough concentration.
Zero order kinetics:
It is possible to detect that the patient has reached zero order by checking concentration after 3 expected half-lives have passed.

Acetaminophen - 10-20 µg/ml, $T_{1/2}$ = 3 hours but decreases to 6.5 hours when toxic doses are ingested. If levels get above 200 µg/ml there is significant probability of acute hepatic disorder, and patient should get acetylcysteine.

Aspirin - 15-30 mg/dl, $T_{1/2}$ = ~ 15 minutes because it is hydrolysed to salicylate which has a half life of 2-3 h at low dose and 15-30 h at high dose. Levels over 50-60 mg/dl are toxic. Toxicity includes respiratory alkalosis (increased) and metabolic acidosis (esp. in children). Treatments include vomiting, charcoal, bicarbonate and if severe hemodialysis. Trinders = Fe^{+3} in acid to give colored complex, with mercuric chloride (to precipitate protein).

Digoxin - 0.9-1.6 ng/ml, T1/2 = 42 ±19 hours no samples prior to 8 hours because in 2-3 hours peak serum but 6-10 hours for tissue peak. Quinidine increases digoxin levels, digoxin like substances cross react, digibind gives mixed results which are assay dependent and the half life for clearance of digoxin-digibind complexes is about 18 hours.

Procainamide - 4-10 µg/ml, $T_{1/2}$ = 3 ± 0.6 hours, is metabolized in thliver to N-acetylprocainamide or NAPA, which is active but different, **Proc + NAPA** - 5-30 µg/ml, $T_{1/2}$ = 6-8 hours. Long term treatment is associated with autoimmune phenomena (~50% have a postivie ANA and 20% of those with ANA have Lupus)

Quinidine - 3-5 µg/ml, $T_{1/2}$ = 6.2 ± 1.8 hours, toxic greater than 8 µg/ml

Lidocaine - 1.5-5 µg/ml, $T_{1/2}$ = 1.8 ± 0.4 hours (also a local anesthetic) and toxic above 9 µg/ml

Theophylline - 10-20 µg/ml, $T_{1/2}$ = 6-8 hours, (methylxanthines) toxic above 25-30 µg/ml, THC and tobacco decrease while antibiotics, cimetidine and chronic liver disease increase theophilline levels. Theophylline may be metabolized to caffeine especially in neonates (~25%).

Caffeine - similar to theophylline, used for neonatal apnea

Penytoin (dilantin) - 10-20 µg/ml, **zero order**, apparent $T_{1/2}$ = 18-30 adults, 12-22 hours in children, toxic over 30 µg/ml

Phenobarbitol - 15-40 µg/ml, $T_{1/2}$ = 87±7 hours, and CNS depression over 40 µg/ml, patients develop tolerance with chronic use (asymtomatic at "toxic doses").

Primidone - 5-12 µg/ml, $T_{1/2}$ = 8±5 hours, is a barbituate, metabolized in the liver to phenobarbitol and PEMA (active but of questionable significance) both with longer half lives than primidone (need to measure both, especially for compliance, e.g. normal primidone with a low phenobarb is indicative of recent resumption of therapy after a long period of non-use).

Carbamazepine or tegretol - 8-12 µg/ml, $T_{1/2}$ = 27±4 hours, microsomal drugs (including carbamazepine) reduce $T_{1/2}$. Active metabolite is the epoxide which is especially dangerous in children.

Valproic acid or depekene - 50-100 µg/ml, $T_{1/2}$ = 16±3 hours, does have an active metabolite but it doesn't accumulate in plasma. It will elevate phenobarbital, and decrease dilantin but keep the same effect of dilantin and drugs that induce hepatic, oxidative enzymes reduce valproic acid concentration.

Ethosuximide or zarontin - 40-100 µg/ml, $T_{1/2}$ = 33±6 hours, (toxicity rare and patients become tolerant to symptoms)

Tricyclics - imipramine, nortriptyline, amoxapine, antidepressants, active metabolites must be measured, concentrations are 10 - 100 times lower than other drugs (50 ng/ml - 500 ng/ml). Response is linear with dose except for nortriptyline where low concn's worsen mood, half lives can be long (10-50 hours). Method of choice is GC-MS, GC with nitrogen/phosphorous detector (common) or HPLC (problems but used).

Lithium - 0.5-1.5 mmol/L, $T_{1/2}$ = 22±8 hours, and 48-72 hours for tissue pool, therefore even after dialysis can get high levels for days.

Vancomycin - 10-30 µg/ml, $T_{1/2}$ = 5-6 hours, does not normally cross the blood brain barrier, not absorbed orally (I.V. or I.M.)

Gentamicin and tobramycin - 2-8 µg/ml, $T_{1/2}$ = 2-3 hours, also not absorbed orally, inactivated penicillin, so must freeze specimen.

Amikacin - 10-25 µg/ml, $T_{1/2}$ = 2 ±0.6 hours, also not absorbed orally, not inactivated by penicillin.

Methotrexate - folic acid antagonist that inhibits DNA synthesis. Common approach cancer chemotherapy is to give a high dose and rescue with leucovorin. Therefore you need methotrexate levels to determine leucovorin treatment. >10 µmol/L after 24 hours, > 1 µmol/L after 48 hours and > 0.1 µmol/L after 72 hours are toxic. Salicylates increase methotrexate. Half life decreases as concentration decreases <0.1 µmol/L = >10 h , <100 µmol/L = 8.4 h, and >100 µmol/L = 1.8 h. Keep urine alkali as pKa = 5.5 so don't get solubility problems and renal failure. Measured by RIA (has metabolite but not important for toxicity).

Cyclosporine A - range varies with organ and times post transplant but generally +metabolites = 250-800 ng/ml whereas alone its 100-150 ng/ml. polypeptide, found about equally in RBC and plasma, with plasma 90% bound to lipoproteins, selectively inhibitory to T-helper cells which lessens both T and B cell responses, oral absorption is variable, toxic to renal tubules.

CLINICAL TOXICOLOGY:

Suggestions of poisoning:

a) low blood oxygen saturation - methemoglobin or carboxyhemoglobin
b) hyperventilation - salicylate
c) ketosis without acidosis - isopropanol
d) high osmole and ion gap - alcohols
e) metabolic acidosis with occult ions - cyanides, methanol, ethylene glycol, lactic acidosis, ox-phos inhibitors

Antidotes:	**Toxicants:**
N-acetylcysteine (Tylenol)	Acetaminophen
Atropine	Organophosphate

pesticides	
Desferoxamine	Iron
Digibind(FAB-fragments)	Digoxin
Dimercaprol (BAL)	Arsnic, Hg
Ethanol	Methanol,
ethylene glycol	
Ethylene Diaminetetracetate	Copper, Lead,
Uranium	
Naloxone	Opiates
Nitrites and sodium thiosulfate	Cyanide
Oxygen	CO
Penicillamine	Cu, Lead
Physostigmine	Anticholinergics
and tricyclics	

Poisoning:

Acetaminophen:

1st 24 hr = nausea, vomiting and diaphoresis (sweating), no acute distress

24-72 hr = improvement hepatic enzymes (ALT, AST) increase, RUQ abdo. tender

3-7 days = jaundice, hepatic necrosis, hypoglycemia, increased protime) hepatic encephalopathy, possible renal failure.

Hepatotoxicity due to reactive intermediate (N-acetylbenzoquinoneimine). At doses greater than 3 g conjunction (elimination) mechanisms are saturated (glucuronide 45-55%, or sulfate 20-30%) or P-450 oxidation 15-20%, then glutathione + intermediate = mercapturic acid, depletes glutathione stores therefore since glut and sulf are saturated more goes through P-450 resulting in more intermediate), intermediate forms covalent bonds (acylation) with cellular molecules resulting in liver damage.

Normal half life is 3-4 hours but as it gets longer hepatotoxicity increases.

Drugs identified by urine metabolites:

Cocaine - benzoylecgonine
Diazepam - oxazepam
Heroin - morphine
THC - THC carboxylic acid

Alcohols:
- methanol is oxidized to formaldehyde which is oxidized to formic acid (blindness), therapy include ethanol, folate, hemodialysis
- isopropanol (rubbing alcohol) is metabolized to acetone, therapy includes hemodialysis but NOT ethanol

head space analysis by GC, dilute with sodium chloride to increase vapor pressure

Barbiturates:
- three ketone groups, 2 nitrogens and R1 and R2
- for long acting barbiturates such as phenobarbital alkaline urine helps ionize the acidic compound and enhance elimination
- analysis by GC

Carbon Monoxide:
- toxic effects are a result of hypoxia (binds hemoglobin 200 times more strongly than oxygen)
- carboxyhemoglobin generally correlates to clinical symptoms
- treat with 100% O_2 or better yet hyperbaric oxygen (less time exposed the less chance of neurological symptoms developing later
- can be measured by spectrophotometric (OK except as at very low CO % - 2-3%) or GC methods

Cyanide:

- CN^-, also prussic acid, binds to heme iron (Fe^{+3}) and uncouples oxidative phosphorylation (in mitochondria)
- treat with sodium nitrite to cause formation of methemoglobin with avidly binds and clears CN- and thiosulfate (a sulfate donor) to enhance clearance via metabolism.

- nitroprusside treatment should be monitored with SCN- since nitroprusside binds hemoglobin in RBC's and would confuse measurement of blood CN-.

Ethylene Glycol:
- antifreeze, itself relatively nontoxic, is metabolized to **oxalic acid** and glycolic acid (major metabolite)
- short half life (~3 hours)
- therapy includes ethanol and bicarbonate and hemodialysis

$-(CH_2)2-(OH)2$

Cannabinoids:
- THC is lipophilic, and metabolized to THC-COOH
- urine may test positive (>15 ng/ml) 2-5 days and up to 10 days post use
- detection in urine indicates past use not immediate
- to monitor compliance use creatinine ratio and an increase of 50% over past value implies reuse
- problem with passive inhalation which is reduced by uping the cutoff to 20 ng/ml
- GC method alkalinizes and derivatizes

Cocaine:
- originates from the E. coca plant
- free base cocaine or crack is smoked and HCl salt is snorted
- metabolized to ecgonine methyl ester and benzoylecgonine methyl ester (converted to ethyl-ester in the presence of ethanol, active like cocaine)

compound	**half life**	**detection (urine)**
cocaine	0.5-1.5 hr	8-12 hours (chronic users 4-5 days)
Benzoylecgonine	4-7 hr	1-3 days (chronic users 10-22 days)

Opiates:

- cause respiratory depression
- treatment for overdose includes opiate antagonist naloxone (Narcan)
- long term treat with methadone (opiate like substance) and wean from it

- heroin is favored because of rapid onset of action (heroin itself is inactive its morphine and 6-acetylmorphine that are active)
- codeine can be converted to morphine and is therefore active as an analgesic

compound	**half life**	**detection (urine)**
heroin	<6 min	(as morphine) 1- 4 days
morphine	1-8 hr	1-4 days, half life elimination is 3-10 hrs, 10% morphine, 50-75% as glucuronide
6-acetylmorphine	1-8 hr	<24 hours (8 hours?)

- can't measure codeine/morphine ratios (poppy seeds and codeine in cough medicine) or 6-acetylmorphine (fast elimination)

PCP
- phencyclidine, renal excretion enhanced when acidic and reduced when alkaline
- a frequent user may excret it for 7-30 days post use
- 65% is excreted as hydroxylated conjugates

Note: Typical detection limit for TLC (Toxi-Lab) for drugs of abuse = 100 µg/dL or 1 mg/L

Metals:

(Note: Spot tests [or **Reinch** test-depends on copper displacing from solution elements below it in the electrochemical series] are considered obsolete because they are prone to yeild false positive results)

Aluminum
- accumulates in bone and brain, especially in dialysis patients
  (need aluminum free water) decreases PTH, treat with deferoxamine)

Arsenic
- common in insecticides
- As+3 is most toxic then As+5 then metabolites
- monomethyl and dimethyl (MMA and DMA) metabolites (partial toxicity)
- organic arsenic in shellfish and cod and haddock are nontoxic (separate first or use HPLC)
- treat with BAL (chelator), action via sulfhydryl binding

Cadmium
Chromium - Cr(VI)
Cobalt - only when consumed as a liquid
Copper - copper sulfate
Iron - hemosiderosis leading to hepatic cirrhosis
Lead - whole blood > 30 µg/dl = significant exposure > 60 = chelation treat with BAL, can also measure RBC protoporphyrin levels (>60 µg/dl)

Manganese - Parkinson-like syndrome, measured by electrothermal a.a. spectro.
Mercury - $Hg^0 < Hg^{2+} < CH_3Hg^+$, order of increasing toxicity, from fish and dental amalgams, treat with BAL or penicillamine.
Nickel - elemental nickel is harmless but $[Ni(C0)_4]$ nickel carbonyl **very** toxic
Platinum - cis-platinum
Selenium - essential element, toxic only in acute overdoses
Silicon - implants
Silver - burn patients and nasal decongestant
Thallium – bi-product of lead smelting

ENZYME KINETICS:

- an enzyme is a protein catalyst
- K_m = Michaelis constant, a large K_m means the reaction is not likely to go while a small Km indicates it is likely to proceed
- Michaelis Menten equation relates reaction velocity to substrate concentration $V = V_{max} \times S / K_m + S$ (note: for zero order or where the amount of substrate won't affect the rate of reaction, and V = 1/2 Vmax then $K_m = S$)

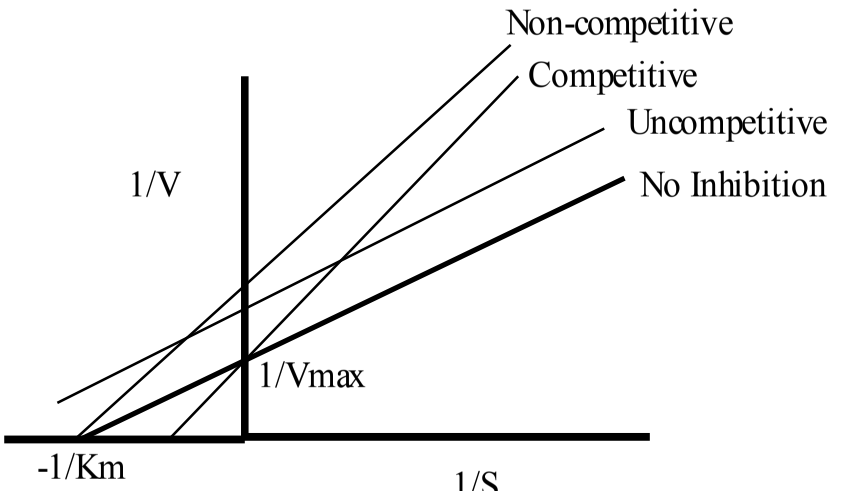
- **Lineweaver-Burke** plot linearizes the velocity vs substrate plot
- for this plot the Y intercept = $1/V_{max}$ and X intercept = $- 1/K_m$
- and the slope = K_m/V_{max}

Competitive inhibitors
- compete for the active site of the enzyme
- similar in structure to the enzyme
- should decrease the rate of the forward reaction and therefore **increase Km** (can overcome if plenty of substrate

Non-Competitive inhibitors
- change some feature of the enzyme but leave the active site intact
- several mechanisms but common feature is that there is no interference with substrate binding with the enzyme active site, therefore Km will be the same but the enzyme doesn't work as well so that V_{max} is decreased. E.G. an enzyme requiring a metal ion such as alkaline phosphatase is inhibited by zinc chelation with EDTA or heat-induced partial denaturation of enzymes

Uncompetitive inhibitors
- combine to form a stable complex with the enzyme substrate complex decreasing both V_{max} and K_m

EFFECTS OF INHIBITORS ON LINEWEAVER-BURK PLOT



Units of enzyme activity

- International unit (IU) = that amount of enzyme which, under the conditions of measurement, catalyzes the conversion of 1 µmol of substrate per minute
- Systeme International (SI) = katal (for catalyst) = that amount of enzyme which, under the conditions of measurement, catalyzes the conversion of 1 mol of substrate per second
(**NOTE: IUPAC and IUB recommend SI units**) Conversion, 1 IU = 16.7 nkat

Measurement of enzyme mass = moles of substrate transformed per second / mole of substrate transformed per second per mole of enzyme

AST - aspartate transaminase (to L-glutamate) (heart and liver ~ equal), in cytosol and mitochondria

ALT - alanine transaminease (to L-glutamate) (slightly more liver specific)
-both are found in plasma, bile, CSF and saliva (NOT urine)

CK - ~ 30-150 U/L (higher in males vs females)
- creatine kinase, BB (CK1) brain, stomach, kidney, MM (CK3) skeletal muscle other, MB (CK2) heart - but to rule in heart it must be more than 5-6% of the total CK, its stable at RT for 24 h, 4¡C for 1 week and -20¡C for 1 month
- sensitivity of mass assay is greater than for activity measurements, serum preferred
- low total CK may lead to apparent elevations in MB due to the lack of precision at low MB values, MB and BB are usually elevated in neonates and young children (heart attack is rare in this group)

LD - ref. values vary greatly but 500 x's greater in RBC etc.,
- lactate dehydrogenase, 4 subunits, 7 isoenzymes (5 commonly analyzed) found in cytoplasm
LD1,2 - **heart**, kidney and RBC (faster moving) (note: flip of 1 & 2 in MI), normally 2 is higher than 1 (hemolysis will show false elevation of LDH1)
LD4,5 - **liver** and skeletal muscle (cathodal)
intermediate mobility - endocrine glands, spleen, lungs, lymph nodes,

platelets
macro form - 2 types, type-1 = CK-1&IgG (could be others) and type-2 = oligomeric mitochondrial CK, both are heatr stable and can interfere with CK-2 analysis

Alkaline Phosphatase –
~40-150 U/L, <500 U/L in <12 year olds
Liver, increased more in extrahepatic obstruction vs intrahepatic
Bone, Paget's disease
Pregnancy - placental form
can separate by PAGE and bone burns (heating), but liver, placental and Regan (placental-like fetal form - in cancers and reacts with placental AP antibody) isoenzymes are heat stable
-urea inhibition, bone least and placental most resistant with liver intermediate found in ER (membranal)

GGT or Gamma glutamyltransferase - < 50 U/L with females less than males, highest concentration in kidney but serum GGT mostly comes from the liver (peptidase found in membrane and cytosol)

Amylase: hydrolyses 1-4 glucose linkages, not 1-6, § amylase is plants, □ is animal also known as endoamylases (random attacks along the polyglucan chain.
Needs calcium and other anions (chloride or...), it is normally found in the urine (the only plasma enzyme normally found in the urine)

ACCR (%) = [U amylase (U/L) x S creatinine (mg/dl) / S amylase (U/L) x U creatinine (mg/dl)] x 100

Starch Based Methods of analysis:
- amylase/creatinine clearance ratios are also frought with false elevations
- problems, differ in composition, deteriorate rapidly, and does not form a true molecular solution but forms hydrated starch micelles
- Amyloclastic Assays - iodometric techniques most popular (uncommon)
- Saccharogenic Assays - reducing sugars made are measured, Somogyi's and Folin-Wu
- Chromogenic - uses dye-labeled amaylase substrates, Phadebas, Kodak, Amylchrome and Abbott

Defined substrates - improves reaction stoichiometry, and controlled conditions means more consistent hydrolysis conditions, small oligosaccaridse e.g. maltopentose, and maltotetrose

Isoenzymes - P3 the best, 12 distinct phenotypes for the salivary and 6 for the pancreatic form

Macroamylase - usually S type, MW > 200,000
Children < 1 year old only have S type amylase

Lipase - needs bile salts and co-lipase for full activity, hydolyzes triglycerides at the 1 and 3 positions but the 2-acylglycerol can isomerize to 3-acylglycerol but it takes time, acts only at the interface between water and the substrate.
- synthesized by the pancreatic acinar cells, elevated 4-8 h to 8-14 days post quicker and longer than amylase

Trypsin - hydrolyses peptide bonds formed by the carboxyl groups of lysine or arginine with other amino acids, acinar cells make trypsinogens1, and 2 (1 is twice as much as 2), converted to active enzyme by trypsin or enterokinase, and is complex by □1 antitrypsin or □2 macroglobulin.

Chymotrypsin - hydrolyzes peptide bonds involving carboxyl groups of Trp, Leu, Tyr or Phe with preference for aromatic groups (vs pepsin which cleaves bonds involving the amino groups of the aromatic amino acids). Is synthesized as 1&2 with 2 being the predominant form, and is more resistant to intestinal proteases therefore is the enzyme of choice for stool analysis.

Cholinesterases - 2 related enzymes hydrolyze acetylcholine
- acetylcholinesterase or true cholinesterase (I), involved with brain (gray) and nerve endings also found in RBC
- acetylcholine acylhydrolase or psuedocholinesterase (II), found in serum, brain (white) etc. (function unknown)
- seru measurements are useful for liver disease, insecticide poisoning and detecting variants, succinyldicholine (suxamethonium) is a drug used in surgery as a muscle relaxant, and resembles acetylcholine. If not removed (as seen with certain variants or low serum cholinesterase) patients may undergo prolonged apnea. Investigated using inhibitors (dibucaine or fluoride)

Acid Phosphatase - pH optimum < 7 for prostate = 5-6, lysosomal forms in all but maybe RBC, extralysosomal form in RBC and others notably the prostate = richest source, but majority of serum activity is from osteoclasts (tartrate-resistant type)
- acidify specimens and keep cool, can differentiate from RBC form using inhibitors, d-tartrate inhibits prostate but not RBC while formaldehyde and cupric ions inhibit RBC but not prostate.

PSA - serine protease, cancerous tissue produces about 10x's as much gram for gram, and females don't produce it, best use is monitoring recurrence post radical prostectomy

GLD - liver mitochondrial enzyme

ICD - primarily liver (but in all) and mitochondrial and cytoplasmic

ACUTE MYOCARDIAL INFARCTION

DIAGNOSIS: A Consensus Document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction has redefined the diagnosis for acute myocardial infarction[1].

Criteria for acute, evolving or recent MI: Either one of the two following criteria satisfies the diagnosis for an acute, evolving or recent MI:

1) *Typical rise and gradual fall or more rapid rise and fall of biochemical markers of myocardial*
necrosis with at least one of the following:

a) ischemic symptoms (chest pain);
b) development of pathologic Q waves on the ECG;
c) ECG changes indicative of ischemia (ST segment elevation or depression); or
d) coronary artery intervention (e.g., coronary angioplasty).

2) Pathologic findings of an acute MI.

Markers: Troponin I, Troponin T, Creatine Kinase (CK), CK-MB, Myoglobin.

Uses Related to AMI:

1) Diagnosis of AMI
2) Assess size of myocardial infarction, especially troponin
3) Determine the success of reperfusion therapy
4) Troponin can be used to risk stratify unstable angina patients (see following)

Blood Sample: Serum or Plasma (assay specific)
Serial sampling recommended as follows[2]:

1) At presentation
2) 2-4 hours
3) 6-9 hours
4) 12-24 (optional)

Reference Ranges:

Troponin I*†	< 0.04 ng/mL (Normal)
Troponin T†	<0.01 μg/L (Normal)
CK-MB*	0.0-5.0 ng/mL
CK*	Male: 35-250 Units/L Female: 25-190 Units/L
Myoglobin*	0.0-110.0 ng/mL

*Reference ranges are laboratory specific
†Troponin upper limit of normal is established as greater than the 99 percentile of a normal population

Test Interpretation:
All Markers: Multiple measurements are required to establish a rise and fall pattern associated with AMI.

Troponin: While troponin I and T are highly specific to the heart they reflect cardiac tissue damage and therefore may be increased in other conditions that cause cardiac damage such as: myocarditis, congestive heart failure, stunned myocardium, PTCA, CABG, cardiac contusion, trauma, sepsis, marathon running, renal failure/chronic dialysis, and stroke. While not common there have been reports of heterophile antibodies interfering with the assay and causing false positive results.
CK-MB: 1% of CK-MB is present in skeletal muscle, colon, ileum, 2% stomach, 6% urinary bladder. Specificity is increased using a **ratio of CK-MB/CK**. Less than 4% is considered normal. Note: CK-MB/CK ratio is independent of test units (no conversion necessary). Also patients with a very low muscle mass may generate an elevated CK-MB/CK ratio in the absence of an AMI. Although controversial the relatively rapid rise and fall in AMI compared to troponin may make this marker more useful for AMI patients that re-infarct while still in house.

CK: Non-specific, reflects muscle damage. Important to calculate CK-MB/CK ratio.

Myoglobin: Non-specific, reflects muscle damage but is presently the only available early marker (note: high sensitivity troponin has shown very high sensitivity and specificity even < 3 hours post chest pain).

Serum Biomarker Changes During AMI

Enzyme	Rise (hours)	Peak (hours)	Return to Normal (days)	Comments
A. Myoglobin	1-2	2-6	1	Early marker, not cardiac specific
B. Troponin	2-12	24-48	5-7	Cardiac Troponin most cardiac specific marker
C. CK	2-12	24-36	3-5	Wide tissue distribution, not cardiac specific
D. CK-MB	2-12	12-24	2-3	Somewhat specific for heart (see above).

RISK STRATIFICATION:

Whether the patient is diagnosed with AMI or not it remains problematic to predict which ones are at high/low risk for adverse outcomes. Risk models using 7-8 patient features are available (TIMI, PURSUIT and GRACE), renal function as measured by the Modification of Diet in Renal Disease (MDRD) equation or cystatin C, and cardiac troponins are all useful for risk stratification in the setting of acute coronary syndrome. Additional tests which may prove valuable include B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) and C-reactive protein[3].

1. The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial Infarction Redefined Journal of the American College of Cardiology 36:959-69, 2000

2. Wu AHB, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes, Jr R. National Academy of Clinical Biochemistry Standardsof Laboratory Practice: Recommendations for the Use of Cardiac Markers in Coronary Artery Diseases.

3. See R, de Lemos JA. Current Status of Risk Stratification Methods in Acute Coronary Syndromes. Current Cardiology Reports 8**:**282–288, 2006.

Liver enzymes - biliary stasis stimulates synthesis of ALP and GGT and elevates total and direct bilirubin, (but if its mostly direct this indicates hemolysis vs stasis). ALP is elevated only if there is biliary stasis. AST and ALT are elevated in parenchymal cell disease

Inborn Errors of Metabolism and Pediatrics:

Disease: Maple syrup urine disease (Branched-chain ketoaciduria)
Smell: caramel or maple syrup (urine)
Defect: branched chain keto acid decarboxylase (deficient)

Disease: Isovaleric acidemia
Smell: sweaty feet

Disease: Phenylketoneuria
Smell: musty
Defect: 5 types, 1-3 phenylalanine hydroxylase (PKU) 1 = classic = absent, types 4 = dihydropteridine and 5= biopterin synthesis defect most common 1/10,000

Disease: Trimethylaminuria
Smell: stale fish

Disease: Hypermethioninemia
Smell: cabbage, rancid butter

Disease: Cystinuria

Screened diseases generally include:

PKU	phenylalanine increased
galactosemia	direct enzyme assay
neonatal hypothyroidism	thyroxine decreased, TSH increased
maple syrup urine disease	leucine, isoleucine, alloisoleucine, valine

Guthrie test - bacterial spores usually B. subtilis are incorporated into an agar medium to which has been added a competitive growth inhibitor specific for the amino acid to be tested. Blood is spotted onto paper and paper put onto plates. the test system is designed to show growth only when the concentration of the amino acid of interest exceeds its upper reference limit. Screens for PKU, MSUD, homocystinuria, tyrosinemia, and hyperlysinemia

Tests include:
a) screening (Guthrie, TLC, and urine color tests)
b) quantitative, for monitoring or confirmation (ion exchange, HPLC, chemical methods)
c) definitive (GC/MS)

For TLC ninhydrin is most common stain and forms a colored product resulting from deamination and condensation, most a.a. become blue or
purple, only the α nitrogen from the original amino acid molecule is part of the measured product.

colorimetric tests includes:
i) ferric chloride - PKU
ii) dinitrophenylhydrazine - ketoacids
iii) nitrosonaphthol - tyrosinemia
iv) nitroprusside - cystinuria

For aminoacidopathies (caused by enzyme defects or abnormalities in a.a. transport systems) plasma is the preferred specimen because the normal range of amino acid levels in plasma is relatively narrow and abnormalities are more easily found.
e.g. PKU, tyrosinemia,urea cycle defects and homocystinuria.

Organic acidemias arise from metabolic abnormalities of physiologic acids that are not a.a. Many are due to more distal blocks in pathways of amino acid metabolism or to accumulation of intermediates of fatty acid oxidation.
e.g. propionic, isovaleric and methylmalonic acidemia

Lysosomal storage diseases are due to deficiences of lysosomal acid hyfrolases which are involved in the degradation of a variety of complex macromolecules, categorized by the type of materia stored in the lysosome.

e.g. - mucopolysaccharides (glycosaminoglycans-polymers of sulfate containing monosaccharides) stored in visceral organs and brain (Hurler syndrome and Morquio syndrome)
- glycoproteinoses (defect due to enzyme necessary for degradation of oligosaccaride chains of glycoproteins, similar symptoms to MPS, e.g. mannosidosis and fucosidosis)
- glycolipidosis (stored material accumulates in neural tissue, GM2 ganglioside in Tay-Sachs or sulfatides in metachromatic leukodystrophy, or visceral and CNS involvement is noted in sphingomyelin accumulation in Niemann-Pick disease)

Peroxisomal (small organelles involved in the metabolism of very long chain fatty acids and plasma membrane ether-lipids also called plasmalogens) abnormalities are grouped into conditions where there are greatly diminished numbers of peroxisomes (Zellweger syndrome), multiple peroxisomal enzyme disorders (chondrodyplasia punctata) and single enzyme disorders (X-linked adrenoleukudystrophy).
Fatty acid oxidation disorders may become apparent only after fasting or a usually benign viral or bacterial illness. Skeletal muscle, cardiac tissue and the CNS can be involved. Mitochondrial beta-oxidation of fatty acids is deficient, e.g. medium chain acyldehydrogenase deficiency (1/10,000) may account for 5-10% of sudden infant death syndrome.

Mitochondrial disorders show maternal inheritance since nearly all mitochondria is inherited from the ovum and are rescessive traits. Examples include MERRF (mitochondrial encephalopathy, ragged red fiber disease) and MELAS (mitochondrial encephalopathy, lactic acidosis, stroke like episodes). Enzymatic diagnosis is difficult and is carried out in research laboratories.

PKU - due to a deficiency of phenylalanine hydroxylase wich converts the essential a.a. phenylalanine to tyrosine. The result is an accumulation of phenylalanine and phenylpyruvic acid and a decrease in tyrosine, causing severe mental deficiency. It affects 1 in 11,000 and less in blacks, is clinicallly treatable with a low diet in phenylalanine. If untreated a child can lose 50 IQ points in one year. Is measured by the Guthrie test (β-2-thienylalanine) and fluorometry.

Galactosemia - caused by a deficiency of:
galactose-1-phosphate uridyl transferase, lethargy, mental retardation, etc.
galactokinase, manifests as cataracts

epimerase, no known symptoms
autosomal recessive trait, 1 in 30,000. Toxicity due to increased galactose and galactose-1-phosphate.
- presence of reducing substances in the urine, I.D. of galactose in serum or urine but definitive diagnosis lies in assay of RBC enzymes, managed by elimination of galactose from the diet

Cystic Fibrosis -most common lethal genetic disease in white population, 1 in 2,000
Autosomal recessive, with heterozygotes unaffected, generalized disease of exocrine glands. Symptoms include thick mucous secretions in many organs associated with chonic obstructive lung disease and persistant infections, exocine pancreatic insufficiency with steatorrhea, cirrhosis of the liver and infertility in males, also a high sweat chloride resulting from failure of salt reabsorption in sweat gland ducts.
Diagnosis made by family history, pancreatic insufficiency, chronic lung disease and the presence of (> 60 mM) sodium or chloride in sweat. 70% of the gene mutations are a loss of a single phenylalanine at 508 of chromosome #7 but > 100 other mutations have been shown.

Molecular Biology:

A - T , 2 H bonds
G - C , stronger 3 H bonds

Purines = A, G
Pyrimidines = T, C

Permissive, low temperature (25-40¡C), high Na+ (>0.5M) and cosolvent present
Stringent, high temperture (65-95¡C), low Na+ (<0.1M) and cosolvent absent

RFLP = restriction fragment length polymorphisms
Estimated 100,000 genes in the human genome

NOTES: