TABLE OF CONTENTS

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

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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	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 or 6-7979 930-6771	7650A 3725
Chemistry Special Chemistry / Electrophoresis	4-5680 4-2854	SW S266
Coagulation	4-5385	SW S288
Diagnostic Molecular Laboratory	4-0452	SW S212
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	4-4865	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)

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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 ~3 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 1.5 to 2.0 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 Laboratory Medicine 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 8:00 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.

Micro/Immuno/Molecular Biology

Survival Guide

2017-2018

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LEARNING OBJECTIVES

I. General Microbiology

- Acquire knowledge of safety issues in Microbiology including handling of infectious agents, chemicals, recommended biosafety levels, and disposal of hazardous waste
- Understand infection control principles and the importance of collaboration between Infection Control and the Microbiology Laboratory for prevention of nosocomial infections
- Acquire an understanding of basic Public Health principles and the vital interaction between diagnostic laboratories and Public Health Agencies
- Develop knowledge of potential bioterrorism agents, laws defining "select agents", and identify resources for information on bioterrorism agents
- Understand quality control testing, proficiency testing, and quality assurance projects needed for optimum identification of infectious agents in clinical specimens and management of a full service clinical laboratory
- Understand the role of the laboratory in hospital antibiotic usage and management and interactions with Pharmacy regarding reporting of antibiotic susceptibilities
- Understand computerized workflow and reporting systems used in University Hospital and how microbiology data are transmitted to the patient medical record

II. Bacteriology

- Describe the major characteristics of infectious diseases caused by aerobic and anaerobic bacteria including

clinical presentation, transmission, pathophysiology, and epidemiology

- Discuss proper specimen collection, appropriate methods for transportation of specimens, and appropriate plating methods used for optimum detection of bacteria in clinical specimens
- Demonstrate proficiency in reading and interpreting Gram stains
- Describe basic types of plating media and broths used to isolate bacteria from various clinical specimens including 5% sheep blood agar, chocolate agar, MacConkey agar, CNA agar, PEA agar, XLD agar, Thayer-Martin agar, BHI broth, thioglycolate broth
- Describe factors important for optimum recovery of pathogens from blood cultures including optimum volume, timing, and number of cultures to collect and discuss advantages and disadvantages of available blood culture instruments and methods, including BacT-ALERT, Isolator, and blood culture media
- Discuss typical gram stain appearance, colony morphology, hemolysis patterns and key biochemical features of commonly-isolated gram-positive *(Staphylococcus, Streptococcus, Enterococcus, Bacillus, Corynebacterium, Listeria)* and gram-negative *(*Enterobacteriaceae,- *Escherichia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Shigella, Yersinia, Citrobacter, Enterobacter, Serratia, Cronobacter, Edwardsiella, Erwinia, Hafnia, Plesiomonas* as well as non-Enterobacteriaceae *Pseudomonas, Stenotrophomonas, Burkholderia, Acinetobacter, Haemophilus, Neisseria, Vibrio, Campylobacter)* pathogens
- Be able to interpret culture results, identify common gram-positive and gram-negative pathogens, and

determine clinical significance of organisms isolated from various body sites, i.e., blood, CSF, urine, body fluids, wounds, stool, and respiratory specimens

- Demonstrate knowledge of methods for culture and identification of anaerobic bacteria including optimum specimen collection, media used for anaerobic culture, and methods used to generate anaerobic conditions.
- Describe media used for isolation of less common or fastidious bacteria including BCYE agar *(Legionella* spp.), TCBS agar *(Vibrio* spp.), Regan-Lowe or Bordet-Gengou agar *(Bordetella pertussis),* CIN agar *(Yersinia* spp.), MacConkey Sorbitol agar (E. *coli O*157), SP4 agar *(Mycoplasma pneumoniae),* A8 agar *(Ureaplasma* spp.)
- Discuss the advantages and disadvantages of methods used to identify bacteria including automated systems
- (MicroScan, Vitek, Phoenix) and manual methods (API strips, biochemical reactions such as oxidase, catalase, PYR, lactose fermentation, metabolism of glucose and other carbohydrates)
- Discuss the advantages and disadvantages of the MALDI-TOF for isolate identification
- Acquire advanced skills in microscopy including the ability to read and interpret sputum gram stains
- Describe the steps necessary for validation of new testing methods in bacteriology
- Discuss the role of quality control procedures to ensure optimal performance of microbiological media, reagents, and assay kits
- Discuss the advantages and disadvantages of molecular assays versus culture for detection of *Chlamydia trachomatis* and *N. gonorrhoeae* in clinical specimens
- Describe methods for detection and identification of mycoplasmas and ureaplasmas in clinical specimens

- Understand methods of bacterial typing for epidemiological and infection control purposes, including pulsed field gel electrophoresis and PCR-based studies
- Describe characteristics of bacterial pathogens that could be used as agents of bioterrorism including *Bacillus anthracis, Brucella* spp. *and Francisella tularensis*
- Discuss rapid and other non-culture-based testing methods available for diagnosis of disease due to Group A Streptococcus, Group B Streptococcus, methicillin-resistant *Staphylococcus aureus, Clostridium difficile, Streptococcus pneumoniae, Legionella* spp., *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

III. Susceptibility Testing

- List the major classes of antimicrobial agents used to treat bacterial, fungal, viral, and parasitic infections and how they work
- Compare and contrast susceptibility testing methods that may be used in the clinical laboratory including automated and manual broth dilution methods, disk diffusion testing, agar dilution testing, and the Etest, PBP2a test for MRSA and use of MRSA and VRE CHROMagar
- Describe the Disk Approximation Test used to detect a "D zone" and discuss when it should be performed
- Describe the nitrocefin test and how it is used to detect beta lactamase production
- Describe the induced beta lactamase test and when it should be performed
- Describe methods used for screening and confirmation of extended-spectrum beta-lactamases in gram-negative bacteria.

- Describe mechanisms responsible for the following drug-resistant phenotypes: vancomycin-resistant enterococci, methicillin-resistant staphylococci, vancomycin-resistant staphylococci, penicillin-resistant *S. pneumoniae*.

IV. Mycobacteriology

- Discuss the major characteristics of diseases caused by mycobacteria including clinical presentation, transmission, pathophysiology, epidemiology, Infection Control issues and Public Health concerns, including notification of positive culture results
- Describe decontamination/concentration procedures used to process specimens sent for culture of acid-fast bacilli (AFB)
- Describe the staining methods for acid-fast bacilli including fluorochrome and carbolfuchsin (Kinyoun) stains
  Read and interpret fluorochrome- and carbolfuchsin-stained smears
- Discuss the advantages and disadvantages of liquid and solid media used to culture AFB organisms and how the Bactec MGIT is used to process and incubate specimens
- Define rapid grower, scotochromogen, photochromogen, and nonchromogen and provide examples of mycobacteria in each category
- Demonstrate knowledge of GenProbe hybridization probes used for culture identification and which mycobacterial species we use it for
- Discuss safety issues associated with culture of AFB organisms
- Name the primary anti-tuberculosis agents and the most important drugs used in treatment of disease due to *M. avium* complex

- Describe susceptibility testing methods used to detect drug resistance in mycobacteria
- Demonstrate knowledge of reference laboratory methods for mycobacterial identification including 16S rDNA sequencing, HPLC and MALDI-TOF
- Discuss the use of PCR-based tests for rapid detection/ identification of mycobacteria from primary respiratory and CSF specimens.

V. Mycology

- Discuss the major characteristics of infectious diseases caused by fungal pathogens including clinical presentation, transmission, pathophysiology, and epidemiology
- Describe methods for detection of fungal pathogens in clinical specimens including methods for direct examination of specimens (KOH smears, Calcofluor white, wet preps)
- Discuss the benefits and limitations of the following non-culture tests for diagnosis of invasive fungal infections: cryptococcal antigen tests, *Candida* antigen tests, galactomannan EIA, beta-D-glucan, *Histoplasma* Polysaccharide Antigen (HPA)
- Describe appropriate specimen collection and processing methods for fungal cultures
- Become familiar with commonly used plating media for fungal cultures including antimicrobial agents used in primary plates for specimens from nonsterile sites
- Discuss testing algorithms for fungal identification including colony morphology on standard media, the cornmeal agar, slide cultures, special agars (e.g., *Candida* CHROMagar media) and biochemical tests

- Identify *Pneumocystis jirovecii* in respiratory specimens and describe available staining methods for this organism
- Identify the following fungi based on colony morphology and microscopic appearance: *Aspergillus fumigatus, A. niger, A. terreus, A. f1avus, Penicillium spp, Paecilomyces lilacina, Histoplasma capsulatum, Coccidioides immitis, Fusarium* spp, *Penicillium marneffei* and *Pseudallescheria boydii*
- Identify the following fungi based on their appearance in tissue: *Coccidioides immitis, Blastomyces dermatitidis, Histoplasma capsulatum, Pneumocystis jirovecii, Candida spp.*
- List the major classes of antimicrobial agents used to treat fungal infections
- Interpret culture results using morphological characteristics of major fungal pathogens and predict clinical significance of an isolate
- Describe susceptibility testing methods for yeast and fungi and discuss interpretation of susceptibility testing results
- Name the *Candida.* spp that are typically resistant or have reduced susceptibility to azole antifungal agents

VI. Parasitology

- Discuss the major characteristics of diseases caused by parasites including clinical presentation, transmission, pathophysiology, and epidemiology. Describe the life cycles of parasites and the major diagnostic stages for identification of intestinal and tissue parasites, blood parasites, and coccidian organisms
- Describe clinical presentation and identification features for *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*

- Be able to collect blood specimens and prepare thick and thin smears for detection of malaria parasites in peripheral blood and learn the morphological characteristics used to identify them
- Discuss proper specimen collection, transportation of specimens, and processing methods for optimum ova and parasite examinations
- Discuss advantages and disadvantages of preservatives, reagents, and stains used in the ova and parasite examination
- Be able to recognize important morphological characteristics used to identify the most commonly identified pathogenic parasites in stool ova and parasite concentrated smears and permanent smears *(Giardia lamblia, Entamoeba histolytica,* Nematodes, *Taenia* spp.,
- *Cryptosporidium, parvum, Cyclospora cayetanensis,* and Microsporidia)
- Demonstrate knowledge of available immunoassays for detection of parasites and discuss advantages and disadvantages associated with use of these assays
- Gain an understanding of the morphological appearance of parasitic larva, ova or adult worms that may be directly observed in clinical specimens
- Learn important characteristics used to identify common arthropods brought to the Microbiology Laboratory for identification
- Name important anti-parasitic agents and the parasites against which they are effective
- Understand use of the fluorochrome stain for screening for giardiasis and cryptosporidium in fecal specimens

VII. Virology

- Discuss the major characteristics of diseases caused by viral pathogens including clinical presentation, transmission, pathophysiology, and epidemiology
- Describe viral pathogens that cause disease in specific patient populations including children, immunocompromised patients, and transplant patients
- Demonstrate an understanding of proper specimen collection, specimen transportation, and processing methods used for viral culture
- Demonstrate knowledge of tissue culture techniques and cell types used to grow viral pathogens
- Describe the immunofluorescent staining techniques used for identification of viruses grown in tissue culture
- Understand the use of the CMV antigenemia test versus shell vial technique for detection of cytomegalovirus infection
- Understand use of PCR for detection of HSV encephalitis
- Understand use of respiratory panel for detection of viral respiratory infections

VIII. Immunology

- Understand principles and application of immunology instrumentation including nephelometry, turbidimetry, immunoprecipitation, immunodiffusion, latex agglutination, ELISA, ELISPOT, hemagglutination, fluorescence microscopy, chemiluminescence, and flow cytometry for performance of assays to diagnose specific diseases and in overall assessment of immune function
- Describe the principles of serological diagnosis for viral hepatitis infection

- Describe use of ELISA, Western Blot, viral load, CD4 cell count and rapid tests that are used to detect and monitor HIV infection and AIDS. Understand the principle and use of serological tests for detection of syphilis including RPR, VDRL, TPHA, TPPA, and FTA
- Understand the principle and clinical application of RAST, cryoglobulin, complement (C3, C4, CH50), rheumatoid factor, ANA, C-reactive protein, and other autoantibody assays
- Be able to list the important infectious diseases of bacterial & viral etiologies in which measurement of the serological response is the primary means for diagnosis

Residents are expected to:

- Attending regularly scheduled immunology QC meetings
- Being on call for the immunology laboratory during the day. This includes handling calls from bench technologists, clinical house staff, clinicians and serving as liaison on behalf of the laboratories at UAB, TKC, and Highlands hospitals
- Viewing all laboratory testing as indicated on the rotation checklist
- Reviewing and participating in daily QA/QC activities of the section.
- Reading of immunology background papers as distributed by faculty and attending the weekly teaching conferences
- Preparing a short PowerPoint Presentation about one clinical test performed in the Immunology

Laboratory and presenting that short talk to the Section Head

- Keeping a log of all immunology related beeper calls and all Paraneoplastic Panel orders (and calling all positive results to the ordering physician)

IX. Molecular Diagnostic / Cytogenetics Laboratory

- All residents will complete a 20 day rotation in the Molecular Diagnostic Laboratory and a 10 day rotation in the Cytogenetics Laboratory under the direction of Drs. Shuko Harada, William H. Benjamin, and Rong Jun Guo. Cytogenetics laboratory deals with tests for cancer and genetic diseases and will not be discussed anymore in this book. Molecular Diagnostic Laboratory deals mainly with genetic tests for various viral infections.
- Molecular Diagnostics main laboratory is located in room **S212** and has the real time PCR equipment and capillary electrophoresis equipment. Viral load testing on menu includes HIV, HCV, HBV, CMV, EBV and BKV. High risk HPV is detected in cervical specimens using Roche's cobas® HPV qualitative test. LineProbe genotyping of HCV using reverse hybridization is also performed in this room. The room has Spartan RX CYP2C19, which offers point-of-care CYP2C19 genotype tests to guide antiplatelet therapy. Recently, we acquired Illumina MiSeq DX, which will offer multiple gene panel-testing for oncology (both hematology and solid tumor).
- Additional rooms are: **S228** for specimen receiving, extraction, and quantitation laboratory; **S227** for reagent and master mix preparation; **S234** (the Clinical Immunology laboratory) for *Neisseria gonorrhoeae*,

Trichomonas, and *Chlamydia trachomatous* assays; and **S250A** (the Mycobacteriology P3 laboratory) for Mycobacterial and fungal culture identification with GenProbe AccuProbe. LAMP methodology is used for detection of *Clostridium difficile* in the microbiology laboratory.

Residents are expected to:

- Understand major molecular diagnostic laboratory procedures and techniques including the principles of the traditional PCR assay, Realtime PCR assay and various instruments and systems.
- Understand validation procedures for laboratory-developed tests. Understand the legal and economic aspects of molecular biology tests performed for diagnostic purposes which are FDA-approved versus analyte specific reagents (ASR) or "for research use only (RUO)" testing
- Be aware of infectious agents currently detected by amplified nucleic acid assays including C. *trachomatis, Trichomonas,* and *N. gonorrhoeae* (offered in the immunology laboratory), Parvovirus B19, Epstein Barr Virus, Hepatitis C (viral load) and genotype, HIV (viral load), BK virus (viral load), CMV (viral load), HPV (all offered in the molecular diagnostic laboratory), Herpes simplex virus in CSF, *M. tuberculosis* in CSF, *Mycoplasma pneumoniae,* and *Nocardia* spp. determination (send-out to reference labs).
- Understand the correlation of molecular tests results with disease processes by:
    - Previewing the cases with molecular test results, obtaining necessary clinical information, and signing out with faculty

- Attending regularly scheduled molecular diagnostic lab meetings
- Being on call for molecular diagnostic laboratory during the day. This includes handling calls from bench technologists, clinical house staff, clinicians and serving as liaison on behalf of the laboratories at UAB, TKC, and Highlands hospitals
- Reviewing and participating in daily QA/QC activities of the section.
- Reading of molecular pathology chapters in Henry's text book and Leonard's book
- Giving end rotation presentation at Molecular Genetic Pathology Joint Conference
- Taking the end of rotation quiz (50 multiple choice questions)

Reference Materials (recommended reading):

General:

Henry's *Clinical Diagnosis and Management by Laboratory Methods*, 22nd edition, 2011 (VIII Molecular Pathology)
Quick Compendium of Clinical Pathology, 3rd edition, 2014. (Chapter 7. Molecular Pathology)

Microbiology:

Manual of Clinical Microbiology, 10th Edition (2011). Murray et al. ASM Press
Clinical Microbiology Procedure Handbook (2004). Isenberg. ASM Press
Bailey and Scott's Diagnostic Microbiology, 11th Edition (2002). Forbes, Sahm, and Weissfeld. Mosby
A Guide to Specimen Management in Clinical Microbiology, 2nd Edition (1999). Miller. ASM Press
Wadsworth Anaerobic Bacteriology Manual, 6th Edition (2002). Star Publishing
Antibiotics in laboratory Medicine, 4th Edition (1996). Williams and Wilkins
Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard -Sixth Edition; M7 -A6 (2003). NCClS
Performance Standards for antimicrobial disk susceptibility Tests; Approved Standard _Eighth Edition; M2-A8 (2003). NCClS
Performance Standards for Antimicrobial Susceptibility Testing M100-S26 (2015). CLSI
Biosafety in Microbiological and Biomedical Laboratories, 4th Edition (1999). CDC/NIH; US Government Printing Office
Medically Important Fungi, 5th ed. (2011). Larone; ASM Press
Diagnostic Medical Parasitology (1997). Garcia and Bruckner. ASM Press
Practical Guide to Diagnostic Parasitology (1999). Garcia. ASM Press

Fields Virology, 3rd Edition (1996). Fields, Knipe, Howley. Lippincott-Raven
Essentials of Diagnostic Virology (2000). Storch. Churchill Livingstone
Hospital Epidemiology and Infection Control, 2nd Edition (1999). Mayhall. Lippincott Williams and Wilkins
Manual of Commercial Methods in Clinical Microbiology. (2002) Truant. ASM Press

Immunology:

Quick Guide to Autoimmune Disease Serology. By Robin G. Lorenz and Moon H. Nahm
Manual of Molecular and Clinical Laboratory Immunology (8th edition). By Barbara Detrick, Robert Hamilton, and John Schmitz
Henry's Clinical Diagnosis and Management by Laboratory Methods (23th edition)
Immunology and Serology in Laboratory Medicine (5th edition). By Mary Louise Turgeon

Molecular Diagnostics:

Molecular Pathology in Clinical Practice, Debra G. B. Leonard, 2007
CAP Molecular Pathology Resource Guide. October 2013
Stanford Open Curriculum in Genomic Medicine – YouTube
Training Residents in Genomics (TRIG) (http://www.pathologytraining.org/trainees/TRIG_lecture_materials.cfm)
AMP and CAP Webinar series

INFECTIOUS DISEASES DIAGNOSTICS

Introduction

Welcome to Infectious Diagnostics. This rotation consists primarily of consultation for clinical services. There are a lot of tests for uncommon disorders requested on this service, so it is especially important to try to figure out not only whether or not a test is appropriate, but to find out what the clinicians really want to know and then to figure out the best way to give them their answer.

Many tests in immunology involve testing for antibodies to a particular pathogen. A common theme with this type of testing is that you often need two samples to be able to reliably make a diagnosis: an acute sample and then a convalescent sample anywhere from 10 days to 1 month later, depending on the test. The reason is that antibodies to a particular organism may be prevalent in the population. Usually the important question that is being asked is, "is this the etiologic agent of this episode?" To decide if that is the case a rise in antibody titer shortly after the clinical syndrome has a high specificity. This concept is especially important for serological tests which only assess IgG rather than IgM antibodies.

Speaking of antibodies, there is an important difference between an association of an antibody with a disease that has been described in the literature (usually as a handful of cases) and antibodies that have been shown to be a useful test for diagnosing specific diseases in clinical practice. Clinicians confuse these two on a regular basis. It is our job to educate about the clinical usefulness of a particular test.

There are different opinions about everything in medicine. Many calls in laboratory medicine, and especially in infectious diseases, are frustrating because the clinicians will sometimes believe that we are putting up roadblocks which are getting in the way of their patient care by suggesting that a particular test is inappropriate. It is our job to try and convince them that we are really trying to help them to pick the best tests in each particular situation. While it is

true that we are an academic institution and there is some value in getting tests for educational purposes, if the clinical utility of a test has not been validated it is more appropriate to order this test off of a research protocol rather than charging the patient. Another thing to keep in mind is that a test with a sensitivity of 50% (which is in the ballpark for a lot of research tests) is no better than flipping a coin.

Try to put yourself in the clinician's place. Many times they are trying to make a diagnosis in very sick patients for whom they really do not know what's going on. Unfortunately, many clinicians will use the shotgun approach and order everything on the menu to try to get an answer. Then we call them up and tell them that we do not think a test is indicated. This frustrates them because after all, it is their patient and here we are telling them that one or more of the tests they ordered is unnecessary or inappropriate. However, the answer in these situations will probably not be found simply by ordering more tests, if those tests are not capable of telling them what they think they will, or if the test is looking for something that is not consistent with the patient's clinical history.

The other situation you will notice is that some clinicians tend to develop ordering patterns of tests that become almost algorithmic rather than evaluating each case on its own merits. One example of this is ordering a battery of PCR tests on CSF before the cell count differential, protein and glucose are known. This is almost certainly due to the fact that ordering PCRs up front is simply more convenient than waiting an hour or two for the preliminary tests, then deciding what PCRs to get based on those results. In most cases, if the cell count and differential were available instantaneously when the needle came out of the patient's back, the clinician would never have ordered all those PCRs. While this practice is efficient from a patient management point of view, it is not always efficient from a laboratory testing point of view and generally will not result in getting an answer more quickly.

The point to stress is that *we are here to assist the clinicians, not hinder them*. Medicine is a large field: no one can possibly know everything. If a clinician becomes irate, tell them that while we recognize that patient care is their area of expertise, we would ask that they recognize that laboratory testing is ours. We know what a laboratory test can and cannot tell them. We also know which tests are the most likely to get a "hit" and which ones are likely to go nowhere. Many times we can steer clinicians to a more appropriate test that they may not have considered. The goal is what's best for the patient.

The CDC Form

A few tests sent out for microbiology get sent to the CDC in Atlanta. Where you will run into this most often is for the Rickettsial serology test (see separate section). Other examples are generally tests for uncommon diseases like neurocysticercosis. Because of this, the CDC keeps tabs on every test by demanding that any test submitted to them be accompanied by their 2 page form which predominantly deals with clinical history and presentation of the patient. It is the clinician's job, not yours, to get this form filled out. The patient's doctor is the physician of record and is therefore responsible for that form. No form, no test. The easiest way to handle this is to fax the form to the clinician and have them fax it back directly to the send-out lab (975-7171). Interestingly, in some cases where you have just approved a test, the index of suspicion for a disease drops precipitously once you mention the form.

Alert Calls

When one of the labs isolates or identifies certain organisms (like *M. tuberculosis*, acid fast smear positive specimens, positive blood cultures, etc.) the physician is notified by telephone. Usually the technologists do this and you never hear about it. However,

sometimes you will be paged because they cannot find the physician. This most commonly occurs when the patient has recently been discharged or it is after business hours and the clinic is closed or a positive blood culture drawn in the UED. If you know the doctor's name, you can call either the Hospital operator to get the house staff responsible for that service. Alternatively, you can call the physician's office and listen to the answering machine for instructions on how to reach that physician or whoever is covering. Some clinics may be closed but will have an operator who will page a covering clinician for you.

Another thing to try is the medicine clinic. During the day you can call and find out if this is the case. Usually a patient will be attached to one resident and then you can call that resident.

There is always a group of residents in house who should be contacted for any emergency results. For instance, a special case may arise if there is difficulty reporting a Group B Strep positive culture in a pregnant woman who is being screened prior to delivery. There is an OB/GYN resident or chief resident that must take this call. If it is a clinic patient they should still take this call but additional information on what to do might be obtained from Labor and Delivery who are always on call.

If you know what particular service a patient was admitted under, for example, infectious diseases, you can call the hospital operator and ask for the infectious disease fellow on call. When all else fails, page the ambulatory care resident on call. Save this for a last resort because although this is their responsibility, they will have never seen or heard of this patient and they will then be stuck trying to do what you were just trying to do.

Another frequently encountered problem is when a specimen is sent without a clinician's name being attached to it. A good place to start is to call Customer Service. They should be able to help you by finding some information and may actually have the ordering clinician's name. If this doesn't work then usually the

patient is on a floor and you can call the floor and find out who is covering the patient currently.

Classical Microbiology tests

Viral Culture of CSF:
Viral culture is typically a relatively low-yield means of detecting viruses in CSF. When virus does grow, most frequently found are: (1) enterovirus, (2) HSV (poor sensitivity), or sometimes (3) Varicella Zoster Virus (very labile and notoriously difficult to culture).
PCR is more sensitive for enteroviruses, HSV and VZV. In addition, PCR turnaround is superior: 1 day turn-around time (on weekdays) vs. culture times of 3-5 days (HSV, enterovirus) or longer (VZV may require weeks).
If the clinician is using viral culture as part of a shotgun approach to determining etiology, it will rarely be effective. However, in the rare cases of significant numbers of lymphocytes in the CSF or mass lesions in the brain, then CSF viral culture may make sense.
* In general, the best thing to recommend is to wait for the **CSF cell count** and **differential** to come back (which generally only takes about an hour). CSF from viral infections tend to have lymphocytes.
* First analyze the **cell count**, represented as two numbers: total cells and nucleated cells. CSF normally should have very few cells of any sort. For viral etiology, we are interested in nucleated cells (rather than the red cells from blood). However, a traumatic, bloody tap will have one additional white cell for every 500-1000 red cells. Any value over 5-8 nucleated cells (once traumatic white cells are excluded) is considered abnormal.
* If there are enough cells to analyze, consider next the **differential**. Look for a high lymphocyte count. (Remember that the lab will spin down several mL of CSF in order to be able to

count 100 cells; keep in mind also that "80% lymphocytes" means absolutely nothing when there are only 3 nucleated cells.)
* If the cell count and differential are consistent with a viral infection, the appropriate next step is to do PCR tests for the viruses consistent with the patient's clinical presentation. Beware: most of the time ALL of the PCRs have already been ordered – which is rarely the best strategy. HSV PCR can only be sent out if there are greater than 5 WBC/mL. Exceptions can be made for immunosuppressed pateints with less than 5 WBC/mL.

Stool Culture:
Stool cultures are performed to look for enteric pathogens. The one issue that sometimes comes up with stool cultures is that the microbiology lab will not accept stool specimens once a patient has been in the hospital for 3 days. Sometimes, this results in a phone call from an irate clinician wanting to know why this is the case. The reason is because if a patient's GI symptoms were what put him in the hospital, the stool should have been cultured at that time.
This means that any stool culture obtained after 3 days of being in the hospital was performed because symptoms started after admission.
A patient should not get *Salmonella* or *Shigella* while in the hospital. Very rarely, a patient was incubating a case upon admission -- or the even more unlikely scenario of the hospital cafeteria as a source. Therefore, even though it is unlikely, sometimes a clinician will come up with a reason which must then be considered on a case by case basis.

Criteria for accepting Stool for complete O&P:
Giardia and *Cryptosporidium* are by far the most common causes of parasite caused diarrhea in Alabama. Thus orders for O&P are cancelled and clinicians are offered the chance to request a complete O&P after the *Giardia/Cryptosporidium* immunofluorescent test is negative. There are some clinical

scenarios where this should not apply and the complete O&P should be done. The clinicians are required to select one of the reasons below and to enter a residents name when ordering a complete O&P.

1) Significant travel history to an area with higher parasite burden than the US.
2) The order was because the patient saw a worm. They would not see *Giardia/Cryptosporidium* so the immunofluorescence would not satisfy the reason for the request.
3) The order is because of an absolute eosinophilia >400/mL. Protozoa do not cause eosinophilia so again the reason for the order would not be fulfilled by the *Giardia/Cryptosporidium*.

Very immunosuppressed patients should be allowed to get a complete O&P including an AFB stain that would detect *Cystoisospora belli* (*Isospora*), *Cryptosporidium*, *Cyclospora* after the Giardia/Cryptosporidium is negative.

Bacterial Susceptibility Testing:

Depending on the organism identified and what type of specimen that organism was cultured from, susceptibilities are performed by Microscan broth dilution, disk diffusion, or Etest. The results are reported as sensitive or resistant based on standards published by CLSI (Clinical and Laboratory Standards Institute).

Often, a clinician will call and want susceptibility for a drug which was not run. This is usually because they started the patient on empiric antibiotic therapy and they want to continue with that drug, but want to make sure that the organism is susceptible.

In some cases, you can "know" a susceptibility result without actually doing it. For example, if a *Staphylococcus aureus* is resistant to oxacillin or methicillin, this means it has the *mecA* gene and it will be resistant to all cephalosporins as well. Generally, the micro techs know these and will tell the clinicians when they call.

Another possibility is that the particular drug a clinician is asking about may have already been run but not reported. Go to the microbiology laboratory and ask a technologist to look up the results on that patient for you. They can tell if that particular drug was suppressed.

Only certain susceptibility results are released for reasons of antimicrobial stewardship. Laboratory medicine and the infectious disease doctors are trying to steer clinicians to use certain antibiotics for certain organisms rather than letting them use their favorites. There are so many antibiotics out there; we are trying to divert antimicrobial coverage to those drugs which we think they should be using based on susceptibility data compiled by the P&T committee based on the hospital formulary, and to help avoid using the wrong drug on an organism. For these reasons, it is a good idea to either understand why that drug was suppressed, or to check the CLSI

Guidelines, or check with a microbiology attending before releasing one of these results to a clinician.

Finally, a clinician may want susceptibility testing for a drug that you can't know empirically and that was not a suppressed report. Most likely, this means that drug probably shouldn't be used for that particular organism in the first place. In a few cases, a drug is technically appropriate for an organism, but CLSI has no published guidelines for susceptibility. (Susceptibilities are described by a minimum inhibitory concentration [MIC] of drug and/or the diameter of growth inhibition around an antibiotic-impregnated disk. If CLSI has not set a guideline for a drug/organism pair, there is no way to know what concentration or how many millimeters of inhibition are considered susceptible vs. resistant.)

We have E-tests for some non-standard drugs. These are strips of plastic coated with a concentration gradient of antibiotic along its length. This allows the technologists to read a minimal inhibitory concentration for that drug. So if a clinician thinks he can get that

concentration of a drug to an anatomical site, that's their decision. Again, check with Dr. Moser or Dr. Bryan about these.

Molecular (Genetic) Tests for Infectious Diseases:
Genetic tests for infectious diseases is often called a NAT (nucleic acid test) or a NAAT (nucleic acid amplification test) because they often use a gene amplification method (e.g., PCR or transcription mediated amplification).
While many NAT tests can pick up one copy of the target DNA in a reaction, you have to understand what a particular NAT means. For example, culture is more sensitive than any of the amplification tests used for *Mycobacterium tuberculosis*. The sensitivity of NAT on smear negative & culture positive specimens is usually no better than 50 – 60%. But for smear positive specimens, NAT testing of AFB is a good test. Therefore, smear positive specimens at UAB trigger a call to the clinician to determine if PCR for *M. tuberculosis* is indicated. The Alabama State Laboratory will do NAT testing for *M. tuberculosis* on smear positive patients if clinically indicated. Another problem is that DNA, and less commonly RNA, of a pathogen can persist in a person for long periods of time. In the case of *M. tuberculosis* this can be months up to a year after successful treatment. The clinical context of the sample is also important. Contamination is more of a problem with the amplified NAT than nearly anything else in the clinical laboratory, so a positive amplified test that does not fit the patient's clinical picture needs to be questioned. Another example is if EBV is found in the CSF of an immunocompetent patient. This is very difficult to interpret, as latent infections are very common in the population and rarely does an immunocompetent person develop EBV positive CNS lymphoma, which is what the test is designed to diagnose.

Below are commonly ordered special genetic testing and how to handle them.

Parvovirus B19:
Blood is generally the preferred specimen of choice for this agent, although bone marrow can be tested. The procedure used here is classical hemi-nested PCR using gel detection. This test is appropriate for detection of disease in immunosuppressed patients, and is not to be used as screening tests for infection.

HIV-1 RNA Quantitative Test:
The procedure in use at UAB is the Roche COBAS® Ampliprep / COBAS® TaqMan® HIV-1 v2.0 RNA Test. This uses real-time PCR to quantitate HIV-1 RNA over the range of 20 to 10,000,000 copies/mL. The test measures HIV-1 RNA Group M, types A - H and O. Other HIV-1 subtypes N and P, and HIV-2 have not been evaluated. This test is appropriate for diagnosis, prognosis, and monitoring treatment efficiency. It is not intended to be used as a screening test for HIV infection: HIV Antigen and Antibody, 4th Generation Screening test with confirmation by the BIO-RAD Multispot HIV-1/HIV-2 Rapid Test should be performed first. (NOTE – Multispot will be replaced with the BIO-RAD Geenius™ HIV 1/2 Supplemental Assay in late 2016/early 2017).
Specimens should be collected in the large (6 mL) purple top Vacutainer tube containing EDTA. The laboratory will perform this test 3 times a week with an expected turnaround time of 3 – 7 days. Results will be reported as copies/mL, and Log10 copies/mL.
* For **newborns,** it is has been recommended that a HIV-1 DNA be ordered. This is the accepted procedure even though only about 30% of maternally transmitted HIV-1 transmission occurs prenatally (and thus nearly 70% of eventually infected infants will be negative by any NAT at birth). By two months of age, RNA PCR will be positive in 95% of infected infants, and nearly always outperforms the DNA PCR. The DNA PCR requires less sample volume than the quantitative test done in house; thus it is often preferable to the RNA viral load test on infants.

HCV viral load, BK viral load:
HCV viral load also uses the Roche COBAS® Ampliprep / COBAS® TaqMan®; genotyping is done with a line probe assay, Siemens VERSANT HCV Genotype 2.0 Assay (LiPA), which hybridizes the PCR products to NA probes on a nitrocellulose strip.
BK viral load testing is done on the Smart cycler.

HPV Test:
UAB MDL tests for the presence of high-risk HPV genotypes using the Cobas HPV Test on the Cobas 4800 system, a qualitatively reported real time PCR. The test specifically identifies types HPV 16 and HPV 18 while concurrently detecting the other high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The use of this test is indicated to screen patients with ASC-US (atypical squamous cells of undetermined significance) Pap smear results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy. In women 30 years and older, this test can be used with Pap to adjunctively screen to assess the presence or absence of high-risk HPV types. This test is not intended for use as a screening for Pap normal women under age 30, and is not intended to substitute for regular Pap screening.

***Chlamydia trachomatis* and *Neisseria gonorrhoeae* tests including *Trichomonas vaginalis*:**
These assays use Transcription Mediated Amplification (TMA) technology (instead of PCR) for the direct, qualitative detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in endocervical swabs, male urethral swabs, and in female and male urine specimens. The tests may provide evidence of infection with *C. trachomatis*, *N. gonorrhoeae*, or of co-infection with both *C. trachomatis* and *N. gonorrhoeae*. The same specimens can also be used to detect *Trichomonas vaginalis*. Results cannot be used to

assess therapeutic success, since nucleic acids may persist following therapy.

Cytomegalovirus (CMV PCR):
The presence of CMV DNA in plasma as detected by PCR indicates that active viral replication is occurring. The minimum detectable quantity for the quantitative real-time assay is 100 CMV DNA copies per mL of whole blood, with a dynamic range up to 10^{10}.
As always, PCR results should be interpreted in light of all other clinical and laboratory tests. For CMV PCR, the positive and negative predictive values of the test are critical. A positive result does not prove that a patient's clinical manifestations are caused by CMV. However, a negative PCR for CMV performed on whole blood does carry a high probability that the patient does not have a clinically significant infection.
The quant RT-PCR CMV assay directly reports as DNA "copies/mL" in blood – referring to CMV genome copies per mL patient whole blood. This value may assist with clinical decision-making regarding the significance of its detection; Microbiology residents are often questioned about the interpretation of the CMV DNA quantitation. However, though there is now an international standard, the threshold for clinical significance (copies indicating CMV disease) is less well defined at present. Quantitation of CMV DNA levels can also be used to monitor the response to antiviral therapy (ganciclovir). One might expect to see a minimum of a 2 to 3 fold decrease in CMV DNA over the course of a week.

CMV PCR is replacing other CMV detection methods:
* Culture -- CMV is a very slow grower; culture can take more than 21 days to develop the typical morphology (foci of grape-like clusters) and is rarely used.
* Shell vial assay -- uses a pre-prepared cell monolayer and centrifugation; much more rapid, and can turn positive in 1 to 2

days. However, sensitivity has been estimated to be ~75%. Not used here.
* CMV antigenemia test -- immunofluorescent staining of the patients PMNs. Antigenemia is reported as positive cells/200,000 leukocytes. Being phased out in favor of in-house qRT-PCR test on blood (plasma); this test has previously been sent to virology at Children's for immunosuppressed patients.
Note: The qRT PCR test on non-plasma samples (CSF, urine, other body fluids or tissues) is sent to a reference laboratory.

Send out NATs:
As genetic test send out requests have been increasing recently, it is important to investigate if an appropriate test is ordered. In general, following considerations have to be made: 1) check patient's clinical history and indication of the test; 2) study the type of test they are ordering and if the test methodology is appropriate; 3) see if the test result would have an impact on clinical management of the patient. After gathering information, if there are any questions, residents should discuss with attending on molecular pathology service.

Common send out NATs include tuberculosis and herpes simplex virus on CSF. ***H. simplex* NAT** is sent to virology at Children's because of the importance of a short turn-around-time. A group of send out tests targets the agents associated with **central nervous system infections**. These agents include: cytomegalovirus, Epstein-Barr virus, varicella zoster virus, JC virus (human polyoma virus), enteroviruses and *Toxoplasma gondii*. The specimen of choice for these infections is generally cerebrospinal fluid. Another group includes PCR for tick borne diseases: *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum. Ehrlichia chaffeensis* found in the Southeast and *A. phagocytophilum* found in the Northeast and great lakes region.

Another common send out NAT is **16s rRNA gene sequencing (16s ribosomal RNA gene).** This is used on sterile site specimens that no bacteria have been isolated from. Tissue is needed and should be stored refrigerated until no growth is seen on the culture, swabs are not acceptable. Paraffin embedded tissue is acceptable. This is a send out to the University of Washington. The method is to amplify all prokaryotic rRNA gene (i.e., DNA) with primers in the conserved region of the rRNA gene resulting in a 1.5 kb amplicon which is then sequenced. The fungal test is 18s rRNA gene sequencing.

Lyme: There have not been any well-documented cases of seronegative Lyme disease with demonstrable *B. burgdorferi* DNA in the CSF. So, instead of testing for DNA, one should look for antibodies in the serum first (see Serology, Lyme section). Lyme disease's largest endemic areas range from Virginia to Maine and through Minnesota and Wisconsin. Testing for Lyme disease should only be ordered if a STRONG clinical suspicion exists. Alabama has reported 17 cases of Lyme disease in 2014 and 25 cases in 2015. There are currently 6 counties in Alabama that are considered to be endemic – Calhoun, Chambers, Jefferson, Mobile, Shelby, and Tuscaloosa. Consequently, this test **requires** resident's approval.

Tuberculosis: There is a PCR for tuberculosis, which is a very attractive concept because it takes so long to culture the organism and AFB staining is not very sensitive. But the genetic test is expensive and is not very good for smear negative respiratory specimens. The sensitivity on culture positive smear negative specimens is about 50% although the specificity is good. For smear positive specimens, the sensitivity is good, though it is still difficult to stop treatment or isolation because of a negative test. Do not forget the PPD, which is read in 48 hours with reasonable sensitivity in an immunocompetent patient. In reality, the MGIT broth system can grow *M. tuberculosis* in a week or two (compared

to 3-4 weeks with solid media only); many clinicians are not aware of the decreased time to identification of *M. tuberculosis* which averages about 14 days for respiratory specimens. The Alabama state laboratory will do PCR on smear positive respiratory specimens with the right clinical history. PCR can be useful for CSF or pleural effusions. These specimens often have low numbers of organisms and thus take a long time for the culture to become positive and thus even with the low sensitivity PCR is reasonable. Formalin fixed paraffin embedded tissue that does not have a culture or an isolate can be sent for PCR. A positive result is much better than a negative result. In view of the above complexity, this test **requires** resident's approval.

Whipple's Disease (WD) PCR:
Whipple's disease is a rare illness caused by the uncultivable bacteria *Tropheryma whippelii*. The disease is typically indolent and primarily involves the GI tract (small intestine), but can also involve the joints, CNS and rarely the heart (culture negative endocarditis). Most patients present with a history of chronic fever, diarrhea, weight loss and arthropathy. The most common scenario for CNS involvement is in patients that had a more classic WD presentation in the past but were treated with antibiotics that did not cross the BBB. However, it has been estimated that about 15% of WD patients have extra-intestinal manifestations without evidence of current or prior GI disease.

Since the bacterium is not culturable, the diagnosis cannot be based on traditional microbiological techniques. Classically, the diagnosis has been based on small bowel biopsy, but PCR for *T. whippelii* has been developed and there is some data on its use from different biological samples. So, this test can be sent out to Mayo only after a resident approves the send out.
The recommended approach to diagnosis depends on the presentation of the patient:

1. Suspected case of classic WD with GI symptoms
   A. Duodenal/Jejunal biopsy--Do histology PLUS PCR on the tissue
2. Suspected case of WD w/o GI symptoms
   A. Neurologic symptoms
      - Small intestinal biopsy is still preferred (histology plus PCR)
      - CSF PCR can be used as an adjunct to GI biopsy
   B. Arthropathy
      - GI biopsy with histology and PCR on tissue is still preferred
      - Synovial fluid PCR only for cases with negative GI work-up and high suspicion
   C. Culture negative endocarditis
      - No current recommendations

A Few Pearls

1. Most of your requests will be from neurology. Just remember:
   -Whipple's disease is rare and CNS WD is VERY rare!
2. There is NEVER an indication to send peripheral blood PCR for WD!!!!
   -Only case reports of its use in the literature and sensitivity is 40%!!!!
   -If they insist on this, then offer a quarter that they can flip.
3. Since the diagnosis of WD is difficult clinically, as long as you have explained the limitations, the case seems remotely possible, and they are sending the proper samples for analysis, it is reasonable to send this off.

Serologic Tests for Infectious Diseases

Cytomegalovirus (CMV Serology):

CMV infection can be:

1. Primary

2. Latent (mononuclear leukocytes and some organs such as the kidney & heart)
3. Reactivation
4. Reinfection

Primary CMV infection is usually asymptomatic. CMV is the most common congenital viral infection and can cause hearing loss, jaundice, hepatosplenomegaly and mental retardation in this population. The symptoms in the immunocompromised host will vary depending on the population: solid organ transplants/hepatitis, bone marrow transplants/pneumonitis, AIDS/retinitis.

CMV IgM appears during the first week of infection and then will take several months before disappearing. CMV IgM serology is useful in pregnant women and patients with heterophile-negative mononucleosis. Difficulties with measuring IgM serology include:

1. IgM is NOT useful for immunocompromised patients (use PCR)
2. Low IgM titers may persist for years
3. IgM may be present during reactivation of CMV infection
4. High CMV IgG can also compete with IgM and cause a false negative result
5. EBV cross-reacts with CMV

CMV IgG will develop after a couple of weeks and then decreases to a lower level that remains for life.

Please note that CMV NAT is available.

CSF Antigen Tests:

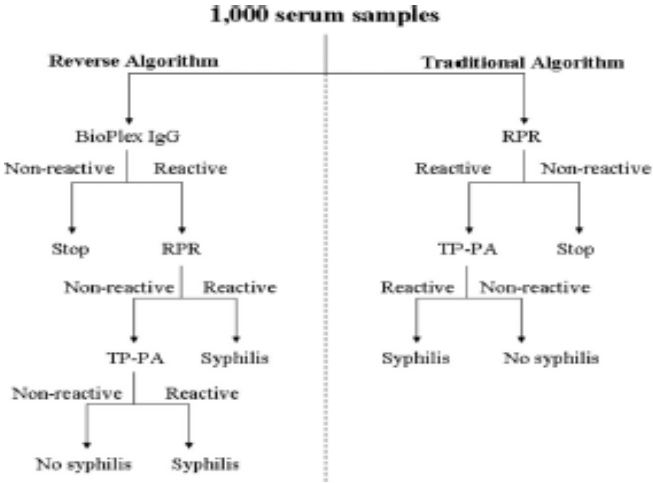
A number of tests for detecting various bacterial or fungal antigens are available as send-outs. Usually the reason a clinician will order one or more of these is to diagnose a "partially treated meningitis". The theory is that if the patient had already gotten some antibiotics, the culture might not grow anything, and so you have to do these tests to make the diagnosis.

Here is the flaw in that reasoning. There is no such thing as partially treated meningitis. It is either appropriately treated or it is not, even if the culture is not growing anything. Yet, if the antibiotic treatment that was begun is appropriate for the organism,

the drug will cure the patient (even if you never find out what the organism is). If the drug is not appropriate for the organism, the culture will eventually grow and tell you what the organism is. This will happen long before you would get any antigen test results back and in addition, many of these tests have poor specificity and poor performance in general.
There is one instance where this type of testing is appropriate. That is in the case of a neonate with suspected Group B Streptococcus meningitis. In this case, testing is appropriate.

Syphilis Serology:
Testing for syphilis is a bit complicated and is commonly done or interpreted incorrectly. It is an issue that comes up all the time. Interpreting a syphilis test incorrectly can result in a patient getting an unnecessary spinal tap, the unnecessary administration of antibiotics, or it could also delay treatment in a case of actual syphilis. Thus, you should memorize the material below.

We changed the order of testing in 2015 and now use the Trep-Sure™, which is an enzyme immunoassay (EIA) for antibodies to treponema antigens, as our screening test. This change was made to aid workflow. The Trep-Sure™ is an automated interfaced test so clerical errors are decreased. We still only want to report patients who are RPR (Rapid Plasma Reagin) positive, thus all positive Trep-Sure™ tests are reflexed to the RPR and/or the older TPPA (Treponema pallidum Particle Agglutination Assay) to confirm. The positive Trep-Sure™ is not reported, only those also positive for either the RPR and TPPA are reported as positive for syphilis.



Non-treponemal tests are used to indicate active cases of syphilis. These tests consist of the RPR and VDRL (Venereal Disease Research Laboratories). These two tests are not specific for Syphilis but have a high sensitivity for active disease. The RPR is the preferred test on a serum sample and the VDRL is the preferred test for a CSF sample. These tests detect antibodies to phospholipid antigens which are present on the outer surface of the spirochete. However, these antibodies are non-specific; and patients with a lupus anticoagulant often exhibit a false positive test for syphilis and patients with syphilis can exhibit a false positive test for a lupus anticoagulant. Non-treponemal tests become positive during acute infection but will generally disappear within 2 years after successful treatment. These tests are good because reactivity parallels disease.

To make sure we do not report false positives, we will confirm Trep-Sure™ positive, RPR negative specimens with TPPA, which uses coated particles to detect antibodies which are specific for treponemes, and it is positive in patients with syphilis but can also be positive in patients with pinta or yaws. TPPA becomes positive during the acute infection (a little bit later than the RPR) but stays positive forever. It generally will never go away.

The reason that we do not report positive Trep-Sure™ results is because it is a specific treponemal test. The problem with reporting a positive treponemal test is that a positive test will only tell you that a patient has had syphilis at some point in their lifetime. IT SAYS NOTHING ABOUT WHETHER THEY HAVE IT NOW. This is why it is imperative to obtain a serum RPR, because although it is only a non-treponemal test, it does parallel disease activity.

A second issue that commonly arises is the appropriate approach to diagnosing neurosyphilis, a late complication of untreated syphilis. A high degree of clinical suspicion is required because neurosyphillis has a very similar clinical picture to other common CNS diseases.
The first step is not a spinal tap. This may be performed anyway in a patient with neurological symptoms, but neurosyphilis testing should NOT begin with CSF. The first test should again be a serum syphilis profile, Trep-Sure™ with positives reflexed to RPR. IF THIS IS NEGATIVE, THE PATIENT DOES NOT HAVE NEUROSYPHILIS. If the RPR is positive, this indicates active syphilis infection. The next test to perform is a VDRL on the CSF. Even though it is a non-treponemal test, a positive VDRL in CSF is virtually 100% specific for neurosyphilis. There is no need to confirm with a spirochete specific test. If, however, the VDRL is negative, there is some literature questioning the sensitivity of VDRL in CSF and so testing by FTA (fluorescent treponemal antibody) might be indicated. This test is requested occasionally

but is generally unnecessary. It is also very expensive, and requires a lot of CSF sample (which could more appropriately be used for other tests, if indicated). Talk to an attending if a clinician is insisting on an FTA on CSF. Another concern is that the FTA on the CSF will be false-positive by the smallest amount of blood (bloody tap).

Arbovirus Serology (West Nile Virus 2008):
WN virus transmission to humans requires a mosquito vector and the normal enzootic cycle involving mosquitoes and birds where viral amplification occurs. Incubation requires 3 to 14 days and most human infections are not clinically apparent. One in 5 persons develop mild febrile illness (3 to 6 days) characterized by fever, headache, weakness, nausea/vomiting, changes in mental status. A rash occurs in half. Serious neurological disease is rare (1 in 150) and encephalitis is more common than meningitis. Diagnosis relies on clinical suspicion: older adults who have onset of unexplained encephalitis or meningitis in late summer or early fall. Advanced age is the most important risk factor for neurologic disease (>50 yrs). Debilitation or immunosuppression may be independent risk factors

Serology for IgG and IgM is performed on serum and CSF. An acute and convalescent sample is preferred. CSF may be the optimal specimen type to diagnose West Nile Virus (WN). WN, California/ LaCrosse and St. Louis Encephalitis (SLE) viruses can be found in Alabama. LaCrosse (California) encephalitis is normally an infection of squirrels and chipmunks; in humans it affects mainly children. St. Louis encephalitis is an infection of wild birds; in humans it affects mainly older adults and shows some serological cross-reactivity with WNV.

Diagnosis of clinically suspected WNV infection is confirmed by a 4-fold increase in WNV-specific antibody titer on sequential specimens; isolation of WNV or detection of WNV antigen or

nucleic acid sequences in clinical samples; or detection of WNV-specific IgM in blood or CSF confirmed with detection of WNV-specific neutralizing antibodies in the same or a subsequent sample.

A positive IgM from CSF indicates a recent infection. A positive IgM for serum indicates infection. WN IgM may remain for more than one year in >50% of patients and thus it is difficult to determine if a positive IgM from serum is from an acute WN infection. 95% of cases from the initial 1999 NY outbreak had positive IgM. IgM does not cross the blood-brain barrier and may persist for 6 months or longer. False positives can occur due to cross reactivity with other flavivirus infections or vaccinations with them.

These ELISA tests detect the host immune response to WNV infection. Although viremia is detectable earlier than the immune response, immunologic (IgG and IgM) assays are typically more sensitive for detecting active and convalescent WNV infection. IgM is typically detectable at the time of initial presentation; IgG can be detected as early as 7 days after illness onset and within 3 weeks of exposure in most infected individuals. Both assays (IgG and IgM) must be performed on the same specimen to help establish whether or not the infection is recent. For suspected neurologic WNV disease, CSF specimens are ideal and should be collected at initial presentation. If only serum samples are to be used for diagnosis, paired specimens should be collected during acute illness and again 7 to 14 days later.

Viral culture of WN is not performed. It has a very low yield and is isolated from blood only during the early febrile stage. Viremia is gone at the onset of symptoms so there are very few positive PCR results for WN. Tests that detect viral antigen also have very low sensitivity.

Toxoplasmosis Serology:
Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*. Infection in adults is usually asymptomatic or associated with a self-limited mononucleosis-like syndrome. Lymphadenopathy is the most common symptom in combination with an array of other symptoms (e.g., fever, malaise). Cats including domestic cats are the definitive host and transmission occurs by the fecal/oral route from cat feces or contaminated soil. Transmission also occurs from raw milk and inadequately cooked meat containing cysts (esp. beef, mutton, or pork). The population in the US has about a 1%/year infection rate. After infection, viable cysts are maintained in the host for life.

Patients on corticosteroids or other immunosuppressive agents and AIDS patients are at great risk for reactivation and can suffer from severe or fatal disease of the CNS. Because of the immunosuppressive regimen given organ transplant recipients, IgG levels are used as a pre-transplant screen. Toxoplasma must be considered in the differential diagnosis of any immunosuppressed patient with clinical or laboratory evidence of damage to the CNS.

Primary infection in pregnant women may cause serious health problems if the parasite is transmitted to the fetus. Congenital toxoplasmosis can have severe sequelae, including mental retardation, blindness, and epilepsy in infancy or later in life. Congenital infections have such a wide range of manifestation that it is usually included in the differential diagnosis of all obscure infant illnesses. Consequently, pregnant women are screened for Toxoplasma IgM and IgG to detect recent infection because the parasite and antigen are rarely detected. Due to specificity problems with this test, the FDA has advised not to use the results from any one Toxoplasma IgM commercial kit. Due to the false-positive IgM results obtained, this test is repeated twice and then sent out for avidity testing to a reference laboratory.

Epstein-Barr Virus (EBV) Serology:
The Monospot Test is used in many ERs and doctors' offices. This is a point-of-care test that assesses the presence of heterophile antibodies, often seen in acute infectious mononucleosis. We just switched to a new Monospot-IgM (Mono II Rapid Test) that only detects IgM, therefore making it more specific for acute disease.

Serology involves 4 types of antibodies:
1. VCA-G, or IgG Abs to the viral capsid antigen
2. VCA-M, or IgM Abs to the viral capsid antigen
3. EA, or early antigen
4. EBNA, or Epstein Barr nuclear antigen

These antibodies appear and disappear at various times in the course of an illness, which is best described in table below. You will sometimes be called to interpret a patient's results based on the antibodies detected.

The antibodies are tested for by ELISA. Borderline ELISA results are confirmed by immunofluorescence. The laboratory does not offer the EA ELISA and the stage of infection is determined using the results from the other three tests using the table below. Another thing to keep in mind when talking to clinicians about these test results is that we do have a PCR for EBV which can be performed on blood or CSF for PTLD. See the PCR section for further details.

Clinical Situation	**Anti-VCA-IgG**	**Anti-VCA-IgM**	**Anti-EBNA**
No EBV infection (susceptible)	Neg	Neg	Neg
Acute infectious mono	Pos	Pos	Neg
Convalescent mono	Pos	Pos/Neg	Pos
Past EBV infection (immune)	Pos	Neg	Pos
Burkitt's lymphoma	Pos	Neg	Pos
Nasopharyngeal Carcinoma	Pos[a]	Neg	Neg

[a]Detected in 75% of cases at very high levels. IgA antibodies to VCA may also be present

Bartonella Serology:
B. henselae and *B. quintana* have both been implicated in bacillary angiomatosis (subcutaneous nodules and fever primarily in HIV or transplant patients), peliosis hepatis, and bacteremia. In immunocompetent patients *B. henselae* causes cat-scratch fever and *B. quintana* causes trench fever.

Cat-scratch disease is characterized by fever, malaise and adenopathy. Important history includes exposure to a cat, especially a kitten, and whether the patient was scratched or bitten.

The test detects IgG and IgM to *B. henselae* by indirect immunofluorescence. A convalescent sample should be drawn in 2-4 weeks. There is also a PCR for *Bartonella ssp.*

Salmonella Serology:
Serology is not a good test for *Salmonella typhi* (typhoid fever) for two reasons. The first is that antibodies to Salmonella tend to be cross-reactive with enteric bacteria which are present in everyone's GI tract. The second is that the vaccines for typhoid cause a positive serology. There are usually less than 500 cases of typhoid fever in the U.S. per year, and most of these are imported. Travel history is a very important part of the workup. The best assessment for non-typhi *Salmonella* infection, which is generally self-limited enteritis, is stool culture. There are about 2400 known serotypes now named for the non-typhi *Salmonella*.

Chlamydia Serology:
The chlamydiae are widespread obligate intracellular pathogens. These organisms produce a variety of infections in mammals and avian species. There are three species known to infect humans: *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, and

Chlamydia psittaci. Serology testing is a send out and is usually ordered for assessing atypical pneumonia for *C. pneumoniae*. Fifteen percent of community-acquired pneumonias are caused by *Mycoplasma pneumoniae*, *C. pneumoniae* and *Legionella spp*. Manifestations of *C. pneumoniae* infection include: pharyngitis, bronchitis, and mild pneumonia. Infections are believed to be commonly acquired between late childhood and early adulthood resulting in a seroprevalence of 40 to 50% in the 30- to 40-year-old age group. Cell cultures for the detection of *C. pneumoniae*, are both difficult and require long incubation times. Serological results can be difficult to interpret because the prevalence of *C. pneumoniae* specific antibodies is high in the general population and because IgM antibodies are often absent in re-infection cases.

Serology can also be used to look for pneumonia secondary to *C. psittaci* in patients who have had exposure to exotic birds. *C. psittaci* is found in domestic mammals and is ubiquitous in bird species, especially, parrots and parakeets. Human contact with secretions, excretions, tissue, and feathers of birds can cause a mild to life-threatening disease called psittacosis. Common symptoms are fever, chills, malaise, headache, and nonproductive cough. Other features include an absence of consolidation and pleural effusion, relative bradycardia, splenomegaly, and a rash resembling the rose spots of typhoid fever. Diagnosis is made clinically. Abnormal results on liver function testing are commonly seen; erythrocyte sedimentation rate (ESR) is usually not elevated; and the chest x-ray typically shows a pattern of nonspecific, patchy infiltrates.

The white cell count is usually normal or decreased. The diagnosis is confirmed by a fourfold increase detected in acute and convalescent antibody titers

Serology testing should not be used for diagnosing the sexually transmitted disease (*C. trachomatis*). We use an in-house amplified

DNA assay. The Hologic Gen-Probe PANTHER system which uses Transcription Mediated Amplification (TMA) assay which simultaneously amplifies and detects target DNA using amplification primers and a fluorescently labeled detection probe. This test is ONLY for endocervical and male urethral swabs and male and female urine specimens. Recurrent episodes of ocular infection by *C. trachomatis* cause progressive scarring of the cornea, leading ultimately to blindness called trachoma. The 18 different serotypes have different tissue tropism and thus disease specificity differs. Serovars A, B, Ba, and C are associated with trachoma, whereas serovars D through K are associated with sexually transmitted (Nongonococcal urethritis and mucopurulent cervicitis) and perinatally acquired infections (inclusion conjunctivitis). Presumptive diagnosis is made by demonstrating a leukocyte predominance on urethral exudate smears with the absence of identifiable organisms on Gram stain. Confirmatory diagnosis is made by either the DNA amplification test or culture. Serovars LI, L2, and L3 are more invasive than the other serovars and spread to lymphatic tissues causing the clinical syndrome of hemorrhagic proctocolitis and LGV (an STD characterized by suppurative regional lymphadenopathy). LVG is not common in the U.S.

Legionellosis (*Legionella pneumophila*) Serology:
Legionellosis is associated with two clinically and epidemiologically distinct illnesses: Legionnaires disease (LD), which is characterized by fever, myalgia, cough, pneumonia, and Pontiac fever, a milder illness without pneumonia. At least 46 species and 68 serogroups have been identified. *L. pneumophila*, a ubiquitous aquatic organism that thrives in warm environments (32°- 45°C) causes over 90% of LD in the United States. An estimated 8000 - 18,000 cases occur each year in the United States; 23% are nosocomial. Most LD cases are sporadic; 10% - 20% can be linked to outbreaks. Pontiac fever has been recognized only

during outbreaks. Legionella is a relatively rare cause of pneumonia in Alabama, but we do see a case now and then, though usually isolated from culture and not *L. pneumophila*. The 2014 cases associated with the Women's and Infants floors 5 and 7 was caused by *L. pneumophila* serogroup 1 with urine antigen positive urines.

Legionella can be diagnosed in the microbiology laboratory by one of the following (revised case definition CDC 1996):

1. Isolation of Legionella from respiratory secretions, lung tissue, pleural fluid, or other normally sterile fluids. Culture may require 14 days for identification and specialized media (buffered charcoal yeast extract agar).
2. Demonstration of a fourfold or greater rise in the reciprocal immunofluorescence antibody (IFA) titer to greater than or equal to 128 against *Legionella pneumophila* serogroup 1. (performed at Quest)
3. Demonstration of *L. pneumophila* serogroup 1 antigens in urine by immunofluorescence, done in UAB microbiology.

Clinicians will normally attempt culture followed by the urine antigen test and then look at serology.

***Helicobacter pylori* Serology:**

Helicobacter species have been isolated from the gastrointestinal and hepatobiliary tracts of mammals and birds. Members of this genus can be grouped as gastric or intestinal (enterohepatic). Gastric *Helicobacter sp.* (e.g., *H. pylori*) possesses several unifying features, including urease activity and the ability to form discrete colonies on plated media. In contrast, intestinal helicobacters (e.g. *H. canadensis, H. cinaedi, H. fennelliae, H. pullorum, H. winghamensis*) inhabit the lower GI and hepatobiliary tracts and have been isolated from rectal swabs and feces. Helicobacter species are microaerophilic and grow poorly, if at all, in routine aerobic atmospheres. They are spiral or curved and are

generally not visible or faint on Gram stain. Intestinal helicobacters have a swarming phenotype and most do not produce urease which is used to help distinguish them from campylobacters. Serological or rapid antigen tests are not commercially available for intestinal helicobacters. Nonselective, blood-enriched media and a prolonged incubation (6 days) are useful for isolation of helicobacters from primary blood culture. Helicobacters often do not stain well with the Gram stain and need a Carbol-Fuchsin counter stain instead of safrin. Rarely are cultures indicated except when treatment failure suggests resistance.
The invasive tests for *H. pylori* include gastric biopsy with histological staining (Warthin-Starry), rapid urease test (e.g. CLO test) on the biopsy itself. The INOVA Diagnostics QUANTA Lite™ *H. pylori* IgG ELISA is offered in immunology and is a semi-quantitative ELISA for the detection of IgG antibodies to *H. pylori* (*Helicobacter pylori*) in human serum. Serum antibody testing cannot differentiate previous from ongoing *H. pylori* infection.

Lyme Disease (*Borrelia burgdorferi*) Serology:
Lyme disease is a nationally notifiable disease and is diagnosed using a combination of a history of potential exposure in an endemic area, recognition of clinical symptoms, and laboratory test results. Lyme disease is a systemic, tick-borne disease with protean manifestations, including dermatologic, rheumatologic, neurological, and cardiac abnormalities. The best clinical marker for the disease is the initial skin lesion (i.e., erythema migrans rash >5 cm) that occurs in 60%-80% of patients.

Testing for Lyme disease should only be ordered if a STRONG clinical suspicion exists. This is not something to order just because someone was out in the woods where there are ticks. Alabama has reported 17 cases of Lyme disease in 2014 and 25 cases in 2015. However, there is controversy about a Lyme-like illness, Southern tick associated rash illness (STARI) that may

exist in the southeast US and is also transmitted by ticks. There are currently 6 counties in Alabama that are considered to be endemic – Calhoun, Chambers, Jefferson, Mobile, Shelby, and Tuscaloosa. In the United States, Lyme disease is mostly localized to states in the northeastern, mid-Atlantic, and upper north-central regions, and to several counties in northwestern California. Ninety-two percent of cases are from the states of Connecticut, Rhode Island, New York, Pennsylvania, Delaware, New Jersey, Maryland, Massachusetts, and Wisconsin.

Alabama currently has a Phase I initiative to determine the incidence of Lyme Disease in these counties. This will be active from April 2017-September 2017. Patients from the 6 counties listed above who are acutely symptomatic or present with clinical signs consistent with early disseminated Lyme Disease should have samples sent to the Alabama Department of Public Health during the acute episode, as well as a convalescent sample collected 3-6 weeks later. Symptoms include Erythema migrans, headache, fever, arthralgia, fatigue, myalgia, cranial nerve palsies, ophthalmic conditions, lymphocytic meningitis, and carditis. Do not send samples from patients who have been previously tested or who have chronic infections. See instructions from the ALDPH for how to send samples and what supporting documentation is needed.

The screening test for Lyme disease is an ELISA. This test detects both IgM (infection or early disease) and IgG (current infection or past exposure). If this test is negative, and the clinician is convinced the patient has Lyme disease because of their clinical history and/or suggestive symptoms, the ELISA may be repeated in 4-6 weeks. Patients in early stages of infection may not produce detectable levels of antibody and antibiotic therapy in early disease may prevent antibody production from reaching detectable levels. Serology is a confirmation test and should only confirm a clinical

diagnosis. There are cross-reactions with EBV, other Rickettsia, and Syphilis.

If patients do not fit the criteria above (i.e. acute infection and from one of the 6 counties), then the ELISA and Western Blot for Lyme disease are sent to Quest:

The Western blot for lyme disease detects IgG or IgM binding to Borrelia *burgdorferi* proteins on a membrane. The interpretation of Western blot antibody assays is based on the number and pattern of band positivity: 2 of 3 bands (23, 39, 41kDa) for IgM positivity and 5 of 10 bands (18, 23, 28, 30, 39, 41, 45, 58, 66, or 93 kDa) for IgG positivity. The Western blot is to be used only following initial EIA testing. According to the CDC, the presence of the 5 or more IgG bands confirms the diagnosis of late Lyme disease infection. This test is NOT useful for confirming a weakly reactive screening ELISA in early infection.

Lyme disease can also have neurological manifestations, and you can see antibodies or DNA in patients who have neurological involvement. However, CSF is not the first place to look for Lyme disease. Look for antibodies in the serum first. There have not been any well-documented cases of seronegative Lyme disease with demonstrable anti-Lyme antibodies or DNA present in the CSF. **IGeneX, Inc. is heavily promoted as a reference laboratory for Lyme disease testing but it is NOT an approved laboratory for Lyme testing**.

Please remember that for Lyme disease, the first thing is patient history. There are very few documented cases of Lyme disease contracted in Alabama (unless you are exposed in one of the 6 counties or have a travel history). Furthermore, the CSF is not the first place to look for Lyme disease. Look for antibodies in the serum first since there have not been any well-documented cases of seronegative Lyme disease with demonstrable *B. burgdorferi* DNA in the CSF.

Rickettsial Serology

Rocky Mountain spotted fever is the most severe and most frequently reported rickettsial illness in the United States. Between 1981 and 1996, this disease was reported from every U.S. state except Hawaii, Vermont, Maine, and Alaska. Over 90% of patients with Rocky Mountain spotted fever are infected from April through September and the highest case rates have been found in North Carolina and Oklahoma. The disease is caused by *Rickettsia rickettsii*, a species of bacteria that is spread to humans by ixodid (hard) ticks. Initial signs and symptoms of the disease include sudden onset of fever, headache, and muscle pain, followed by the development of rash in most cases. The disease can be difficult to diagnose in the early stages, and without prompt and appropriate treatment it can be fatal. The indirect immunofluorescence assay (IFA), which has 94% to 100% sensitivity and 100% specificity is sent to Quest. A negative IFA IgM and IgG result (titer <1:64) does not exclude rickettsial infection; a negative result obtained from a second specimen collected 7 to 14 days later may be necessary to exclude infection if early acute stage infection is suspected. An IgM titer >1:64 is suggestive of current or recent infection, but should be interpreted in conjunction with results from a second specimen collected 7 to 14 days later, especially if the IgG results are negative. An IgG titer >1:64 but <1:256 is suggestive of past or early stage infection, and a second specimen is again needed. A 4-fold or greater increase in titer between first and second specimens strengthens the evidence for recent infection. An IgG titer 1:256 or greater is presumptive evidence of recent or current infection. Cross-reactivity among spotted fever rickettsiae precludes speciation of rickettsiae; thus, this test is not specific for RMSF.

Pneumococcal Titers

ARUP performs testing for antibodies to *Streptococcus pneumoniae*. The only indication for performing this test is to

assess immune function in general. This is ordered primarily in adult patients who have recurrent bacterial infections. The way this test works is to first get a serum sample from a patient, administer the Pneumovax vaccine, then get another serum sample one month later. Both samples then get sent to determine whether the patient initiated an appropriate antibody response. It is important to remember that this is a test of general immune function, NOT a test for Immunity to Pneumococci. *S. pneumoniae* was picked ONLY because there is a readily available vaccine (Pneumovax) to challenge patients with; in other words, it is a convenient model system.

What this test is inappropriately ordered for in many cases is to monitor a patient's response to Pneumovax. The clinician wants to know whether the patient will be protected from infection with *S. pneumoniae*. THIS IS NOT AN APPROPRIATE USE FOR THIS TEST. The reason for this is that the Pneumovax vaccine contains 23 different capsular polysaccharides of the greater than 90 known pneumococcal serotypes. The test only assays for 14, leaving the other 80 or so untested. So it is a useful test to assess general immune competence because you're just looking for any antibody response to a challenge with a polysaccharide antigen. However, if you're trying to assess immunity to *S. pneumoniae,* only testing 14 serotypes leaves a big hole of 80 other serotypes that the patient may still be susceptible to.

There are many times where a clinician still demands a test even though it doesn't seem indicated. In these cases we generally give the clinician the benefit of the doubt and give them the test. THIS TEST IS ONE OF THE FEW EXCEPTIONS TO THIS RULE. It cannot be used to assess susceptibility to infection with *S. pneumoniae*.

HIV1/HIV2 Antigen/Antibody

Our current HIV testing is comprised of a 4th Generation Ag/AB Combo Assay that is used for screening. If this test is repeatedly

positive, then the sample is tested by a Geenius Confirmatory Assay. The table on the next page shows the potential assay interpretations from this Geenius assay. This confirmatory assay differentiates between HIV-1 and HIV-2 infection; however, it only measures antibody to HIV and therefore may be negative in the setting of an acute infection (where only HIV antigen is present, which would make the screen positive). These patients need to be tested for the presence of acute HIV infection by a HIV molecular test.

In this scenario, the Clinical Immunology laboratory or Emergency Room laboratory (for needlestick or OB patients) will contact the LM resident. You should then:

- Contact the ordering provider. Notify them that their patient may have an acute HIV infection and needs to be tested by a HIV molecular test. The ordering provider needs to order the molecular test and have the new sample drawn ASAP. There are several options for HIV molecular tests.
    a. HIV-1 RNA, qualitative (sent out to Quest, requires a Lavender top tube) is the only FDA approved test for diagnosis. However, this is a send out test and takes several days to get results. The result is reported as positive/negative.
    b. HIV-1 Viral Load, Quantitative (done by UAB in Molecular Diagnostics) requires a Lavender top tube. Performed 3-5 times/week. This is only approved for monitoring patients, but appears to have similar sensitivity as the send out qualitative test and gives an actual HIV-1 RNA level. Since this is done in-house, this is the fastest turnaround, but the drawback is that

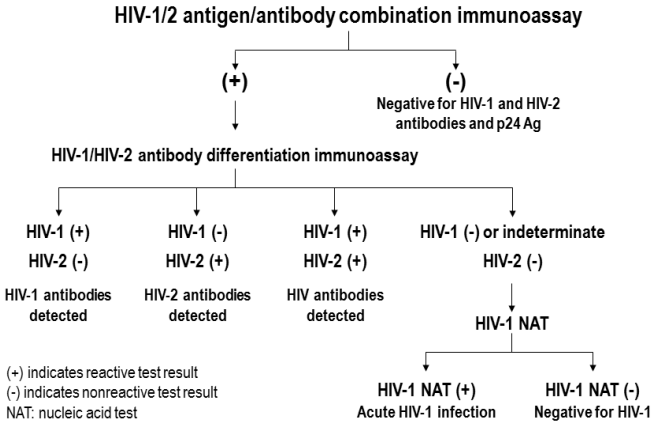
it isn't FDA approved for diagnosis (however, this is what our HIV clinics utilize and prefer).

c. HIV-2 DNA/RNA, qualitative (only order if Geenius testing implicates infection with HIV-2; this is a sendout to Quest). This is also drawn in a Lavender top tube.

- Contact Sonya Heath (ID; pager 8468) or the ID Fellow on call (if Dr. Heath is not available) with the patient information and let them know that there is a patient with a potential acute HIV infection. This can be done the next morning.

NOTE: If the order is on a patient from the 1917 clinic, then just check to make sure they have ordered the molecular testing. If so, then there is no need to call the ordering provider or Dr. Heath.

See the next page for the screening algorithm and the Assay interpretation for the Geenius Confirmation Assay. Call Dr. Robin Lorenz (pager 6787) with questions.



AUTOIMMUNE DISEASES DIAGNOSTICS

Stat Serum IgA

Some IgA deficient persons have antibodies to IgA and react to blood products containing IgA. To avoid such situations, many patients are tested for IgA levels before receiving blood products such as gamma-globulin. IgA assay is currently performed 24/7 in clinical chemistry laboratory. Thus, IgA assay can be ordered anytime. However, you must be aware that our IgA assay is not very sensitive. Thus, when a person has undetectable level of IgA, that does not mean the patient is absolutely IgA deficient. To be certain if a person is absolutely IgA deficient, a more sensitive assay must be used.

ANCA (Anti-Neutrophil Cytoplasmic Antibodies)
Serum antibodies directed toward components of the neutrophil cytoplasm are associated with several disease processes, the most important of which to diagnose early is Wegener's granulomatosis (WG).
An ANCA screening is done by immunofluorescence and the pattern of staining is described as either C-ANCA (C for cytoplasm), P-ANCA (P for perinuclear), or atypical A-ANCA (A for atypical).
The majority of C-ANCA antibodies are directed toward the 29kD serine proteinase 3 (PR3). The C form of ANCA is associated with WG, which is a systemic vasculitis that manifests primarily as renal and pulmonary disease. This disease is rapidly fatal (usually within 1 year) if untreated. The test has sensitivity of 85% in active disease and a specificity of approximately 100% (false positives are extremely rare). In cases of documented WG, the C-ANCA Titer may be used to follow disease activity. C-ANCA is rarely observed in patients with Goodpasture's disease.
P-ANCA antibodies are directed toward various elements within neutrophil cytoplasm, most notably myeloperoxidase (MPO), but also elastase and lactoferrin. These antibodies can be found in patients with other systemic vasculitides. The most important of these is pauci-immune necrotizing glomerulonephritis. The pattern of staining is important because this disease, like WG, has renal manifestations, which can result in misdiagnosis and confusion. P-ANCA has also been documented in patients with **inflammatory bowel disease;** more common in ulcerative colitis but also can be seen in Crohn's disease.
ANCA tests are not so precise and accurate. One should follow up ANCA tests with ELISA assays for MPO and PR3. Also note that anti-TPO antibody may be detected as anti-MPO antibody.

Summary of Different Patterns of ANCA, and Appearance on Differently Fixed Neutrophils

Auto antibody Type	Assoc. Antigen	Pattern on Ethanol-Fixed Neutrophils	Pattern on Formalin Fixed Neutrophils	Test
C-ANCA	Proteinase-3(PR-3)	Granular cytoplasmic	Granular cytoplasmic	PR3-EIA
P-ANCA	Myeloperoxidase (MPO)	Perinuclear	Granular cytoplasmic	MPO-EIA
A-ANCA	Not well characterized	Perinuclear variants	Negative (nonstaining) Or diffuse cytoplasmic	None
ANA FANA on	Various nuclear antigens	Nuclear Or nuclear	Negative (nonstaining)	HEp-2 cells

ANCA, anti-neutrophil cytoplasmic antibody (C, cytoplasmic pattern; P, perinuclear pattern; A, atypical); EIA, enzyme immunoassay; ANA, antinuclear antibody; FANA, fluorescent antinuclear antibody/

Anti-Nuclear Antibodies (ANA)

Antinuclear antibodies are found in a wide variety of autoimmune disorders. Testing is performed by immunofluorescence on Hep-2 cells. The pattern of fluorescence can help determine the specificity of the antibody. When an ANA is ordered, a qualitative ANA and anticentromere test are performed. If positive, a titer can be performed. A titer greater than 1:80 is considered to be significant. It is unclear whether these antibodies are involved with the pathogenesis of disease (probably not) or whether they are simply a marker of the disease (more likely). Therefore, ANA titers are useful only in the initial diagnosis. They are not used to follow the progression of disease.

ENA, or extractable nuclear antigens, are the target of a subset of ANAs that get their name from the fact that these antigens can be

easily extracted from the nucleus. The antibodies to these antigens generally show a speckled pattern of the ANA immunofluorescence.

The disease associations and the diagnostic pathway for ANAs are illustrated in the charts on the next two pages.

Characteristics of Antibodies Detected in Non-Organ-Specific Autoimmune Diseases				
Antibody	**Pattern on FANA**	**Antigen**	**Function**	**Disease Specificity**
Generic ANA	Nuclear	Many nuclear Antigens	Many different functions	Sensitive; not specific for SLE and other auto-immune disease
Anti-dsDNA	Homogeneous	Native dsDNA	Genetic Information	60% SLE, specific for SLE
Anti-histones	Rim	Different classes	Nucleosome Structure	SLE, drug-induced SLE
Anti-Smith (Sm)	Speckled	snRNP proteins	Splicing of pre-RNA processing	30% SLE, Specific for SLE
Anti-U1-RNP	Speckled	U1-snRNP proteins	RNA processing	MCTD, SLE
Anti-SS-A (Ro)	Speckled	Proteins complexed to Y1-Y5 RNA	Unknown	Sjögren's syndrome SLE, neonatal and cutaneous lupus
Anti-SS-B (La)	Speckled	Phosphoproteins	Processing of RNA	Sjögren's syndrome
SCL-70	Nucleolar, atypical	DNA topoisomerase	Relaxation of Scleroderma supercoiled DNA	Scleroderma
Nucleolar	Nucleolar	RNA polymerase I Nucleus organizer protein	RNA polymerase transcription	Scleroderma, SLE
Anti-centromeric	Centromeric (discrete speckled)	Inner and outer kinetochore plate Proteins	Cell mitosis	Crest syndrome scleroderma

ANA, antinuclear antibody; SLE, systemic lupus erythematosus; dsDNA, double-stranded DNA; MCTD, mixed connective tissue disease

Celiac Sprue (Gluten Enteropathy)

Celiac Sprue is an abnormal sensitivity to gluten in the diet that causes chronic diarrhea and abdominal discomfort. **The gold standard for diagnosis is a salutary response to a withdrawal of gluten from the diet.** Alleviation of symptoms is diagnostic of the disease. There are a number of disease-associated antibodies which, while known not to be the pathogenic cause of the disease, are markers of it.

anti-TTG (tissue transglutaminase) antibodies of the IgA subtype. This test is more reproducible and is 95-100% sensitive. Performed by ELISA. Currently recommended as the first line test.

anti-endomysial antibodies of the IgA subtype. They can be seen in patients with disease. Performed by indirect fluorescent antibody assay.

Both of the above tests detect IgA antibodies; therefore, they are unreliable if the patient is IgA deficient. Although IgA deficiency is present in about 1 in 250 in the general population, it is much more common among celiac sprue diseased patients.

If these two tests are negative but the clinical suspicion remains high the other option (besides gluten withdrawal) is the **anti-gliadin antibody test.** This test detects IgG antibodies to gliadin. This test is not recommended as the first line test because it is not as specific for celiac sprue. However, it is the only option beyond gluten withdrawal for diagnosing IgA deficient patients.

Cryoglobulins
Cryoglobulins are immunoglobulins that precipitate at temperatures below 37ºC and dissolve when heated. Cryoglobulins are classified into three characteristic groups:

Type I: Isolated monoclonal immunoglobulins

Type II: Mixed monoclonal immunoglobulins composed of two immunoglobulin components, one of which is monoclonal, possessing antibody against polyclonal IgG.

Type III: Mixed cryoglobulins composed of one or more classes of polyclonal immunoglobulins which form circulating immune complexes.

Types I and II are associated with monoclonal gammopathies, such as lymphomas, Waldenström's, macroglobulinemia, multiple myeloma, etc. Types II and III can be associated with various conditions such as autoimmune diseases and chronic infections (hepatitis C, CMV, EBV).

Classical clinical symptoms include purpura, arthralgias, and renal involvement. Type I primarily presents with Raynaud's phenomenon.

Specimen handling: Cryoglobulin analysis requires the collection of blood into prewarmed (red top) tubes and transport to the laboratory at 37°C. A special device (Cryocab) has been made to transport blood samples at 37 ºC. Cryocabs are available from the Clinical Immunology Laboratory.

Laboratory analysis: The specimen is allowed to clot for 30 minutes at 37°C and spun in a 37°C centrifuge.

The serum is then refrigerated at 4°C; it is examined for the appearance of a cryoprecipitate after 48 hours and 240 hours. If

cryoglobulin is detected this is analyzed by electrophoresis and immunofixation.

Paraneoplastic Antibodies

There are a number of clinical syndromes, mostly neurological, that have been associated with a growing number of antibodies (Abs) expressed as a result of a known or an occult malignancy. The basic principle is that some tumors express antigens normally-expressed in neural tissue and induce antibodies to the neurologic antigens. These Abs then target the CNS and cause of many of these syndromes. These cases can be frustrating to clinicians because real cases are rare but display non-specific symptoms. So, neurologists use the tests to exclude paraneoplastic Abs for patients with bizarre clinical manifestations that don't have an obvious cause. Each of these Abs tends to be restricted to either one or a few types of tumors. For example there's no point in looking for Yo in a male patient this antibody has been described in gynecologic tumors.

UAB generally uses an assay (Test ID: PAVAL: Paraneoplastic Autoantibody Evaluation, Serum; Cost to UAB $370) performed by the Mayo laboratory. For more information, see Mayomedicallaboratories.com test catalog (explains the testing algorithm and methods). Of note some of the antibodies listed on the panel are tested by looking at the immnoflourescent patterns produced using cell binding assays. If the immunofluorescent staining pattern is indicative of expression of other non-panel antibodies (ie NMDA-R, GABA-B, AMPA-R and Gad65), tests for these antibodies will be performed for an additional charge (range of costs for the additional reflex testing is $25 - $450). Of note-Mayo reports this is not the most sensitive way to approach testing for these additional antibodies. Mayo offers several other panels:

DEMES – Dementia, Autoimmune Evaluation, Serum (Cost ~$1400)

ENCES – Encephalopathy, Autoimmune Evaluation, Serum (Cost ~$1400)

EPIES: Epilepsy, Autoimmune Evaluation, Serum (Cost~$1400)

One additional note this test should not be ordered for any patient who has received IVIG therapy. IVIG treatment invalidates test results in both serum and CSF for 6-8 weeks. The test is also invalid after we have done therapeutic apheresis, so if the team thinks they might eventually want to send the test, then the sample should be drawn and held (serum is stable for 28 days if refrigerated).

Antibody (Serum)	PAVAL	DEMES	ENCES	EIPES	Available to Order Separately
Useful For	Subacute neuro-logical disorder of undeter-mined etiology	New onset dementia	New onset enceph-alopathy	New onset crypto-genic epilepsy	
Anti-Neuronal Nuclear Ab (ANNA), Type 1, 2, 3	X	X	X	X	
Anti-Glial Nuclear Ab, Type1	X	X	X	X	
Purkinje Cell Cytoplasmi c Ab Type 1, 2, Tr	X	X (2, Tr only) (1-Reflex)	X	X (2, Tr only) (1-Reflex)	
Amphi-physin Ab	X	X	X	X	
CRMP-5-IgG (CRMS)	X	X	X	X	Yes (as WB-SRMWS)

Striational (Striated Muscle) Ab	X				X (STR)
P/Q-Type Calcium Channel Ab	X	X	X	X	
N-Type Calcium Channel Ab	X	X	X	X	
ACh Receptor (Muscle) Binding Ab	X	X	X	X	X (ARBI)
AChR Ganglionic Neuronal Ab	X	X	X	X	
Neuronal (V-G) K+ Channel Ab	X	X	X	X	
GAD65 Ab	Reflex	X	X	X	X (GD65S)
NMDA-R Ab CBA	Reflex	X	X	X	
GABA-B-R Ab CBA	Reflex	X	X	X	
AMPA-R Ab CBA	Reflex	X	X	X	
NMO/AQP 4 FACS	Reflex	Reflex	Reflex	Reflex	X (NMOFS)

Antibody (CSF)	PAC1	DEMEC	ENCEC	EPIEC	Available to Order Separately
Anti-Glial Nuclear Ab, Type 1	X	X	X	X	
Amphi-physin Ab	X	X	X	X	
Anti-Neuronal Nuclear Ab (ANNA), Type 1, 2, 3	X	X	X	X	
CRMP-5-IgG	X	X	X	X	Yes (as WB-SRMWC)
Purkinje Cell Cytoplasmic Ab Type 1, 2, Tr	X	X (2,Tr only) (1-Reflex)	X	X (2,Tr only) (1-Reflex)	
Gad-65	Reflex	X	X	X	Yes (GD65C)
NMO/AQP4 FACS	Reflex	Reflex	Reflex	Reflex	Yes (NMOFC)
NMDA-R Ab CBA	Reflex	X	X	X	
AMPA-R Ab CBA	Reflex	X	X	X	
GABA-B-R Ab CBA	Reflex	X	X	X	
VGKC-complex Ab IPA	Reflex	X	X	X	

As is clear from the table above, these assays almost completely overlap with each other and therefore ONLY ONE of these tests should be approved per patient.

Mayo also offers a similar set of panels for CSF Testing. PAC1: Paraneoplastic Autoantibody Evaluation, Spinal Fluid (Cost~350); DEMEC: Dementia, Autoimmune Evaluation, Spinal Fluid (Cost=$900); ENCEC: Encephalopathy, Autoimmune Evaluation, Spinal Fluid (Cost=$900); EPIEC: Epilepsy, Autoimmune Evaluation, Spinal Fluid (Cost=$900)

Many of these same assays are used in the panels to diagnosis Myasthenia Gravis/Lambert Eaton Syndrome. Mayo offers multiple panels. MGL1: Myasthenia Gravis (MG)/Lambert-Eaton Syndrome (LES) Evaluation MGA1: Myasthenia Gravis (MG) Evaluation, Adult; MGT1: Myasthenia Gravis (MG) Evaluation, Thymoma MGRM: Myasthenia Gravis Evaluation with MuSK Reflex, Serum

Antibody (Serum)	MGL1	MGA1	MGT1	MGRM	Available to Order Separately
Useful For	MG vs. LES	DX of MG (initial eval)	Suspected thymoma (with or without MG)	MG with MUSK (useful if AChR binding and modulating antibodies are negative)	
CRMP-5-IgG (CRMS)	Reflex (WB)	Reflex (WB)	X (WB)	Reflex (WB)	Yes (as WB-RMWS)
Striational (Striated Muscle) Ab	X	X	X	X	X (STR)
P/Q-Type Calcium Channel Ab	X				
N-Type Calcium Channel Ab	X				
ACh Receptor (Muscle) Binding Ab	X	X	X	X	X (ARBI)
AChR Ganglionic Neuronal Ab	Reflex	Reflex	X	Reflex	

Neuronal (V-G) K+ Channel Ab		Reflex	X	Reflex	
GAD65 Ab		Reflex	X	Reflex	X (GD65S)
NMO/AQP 4 FACS					X (NMOFS)
ACh Receptor (Muscle) Modulating Ab	X	X	X	X	
MuSK Autoanti-body				Reflex	X (MUSK)

If the patient is being evaluated for MG/LES then the more limited, specific (and cheaper) MGL1 panel should be ordered.

Some additional names for the assays and their assay types are (ANNA- l (anti-Hu), ANNA-2 (anti-Ri) ANNA-3, PCA-1 (anti-Yo) PCA-2, PCA-tr (anti- tr), AGNA-1 , ampiphysin t\b, and CRMP-5-IgG) are done by IFA. Five assays are RlAs (P/Q type Ca Channel Ab (Anti-VGCC), N-typc Ca channel Ab, muscle AChR Ab, Gagl ionic AChR Ab, and Voltage gated K channel (VGKC) Ab) and one is an ELlSA (Striated muscle Ab).

The table below outlines the most well-known paraneoplastic autoantibodies, the clinical presentations associated with them, and

their most commonly associated cancers. One may use the clinical presentation to suggest a specific antibody test. Cancer-associated retinopathy (CAR) is a paraneoplasic blinding disease and is due to anti-recoverin autoantibodies that have been shown to induce apoptotic death of photoreceptor cells. One particular antibody, Abs to K channels, is associated with Isaac's syndrome (neuromyotonia- a very rare disease). It is not an orderable test, but Mayo will do it on a research basis IF a comprehensive panel is ordered.

http://www.neurocast.com/site/content/sessions_archived_paraneoplas

Autoantibody	Clinical Presentation	Commonly Associated Cancer(s)
Anti-Hu	Encephalomyelitis, sensory neuronopathy	Small Cell Lung Cancer (SCLC) and neuroblastoma
Anti-Ma	Cerebellar dysfunction, Brainstem dysfunction	Breast. Lung, colon[7]
Anti-Ta	Limbic encephalitis, Brainstem dysfunction	Testicular
Anti-Yo	Cerebellar degeneration	Gynecologic, breast
Anti-Ri	Cerebellar ataxia, Opsoclonus	Breast, gynecologic, SCLC
Anti-CAR	Photoreceptor degeneration	SCLC and others
Anti-VGCC	Lambert-Eaton Myasthenic syndrome	SCLC

Recommendations:

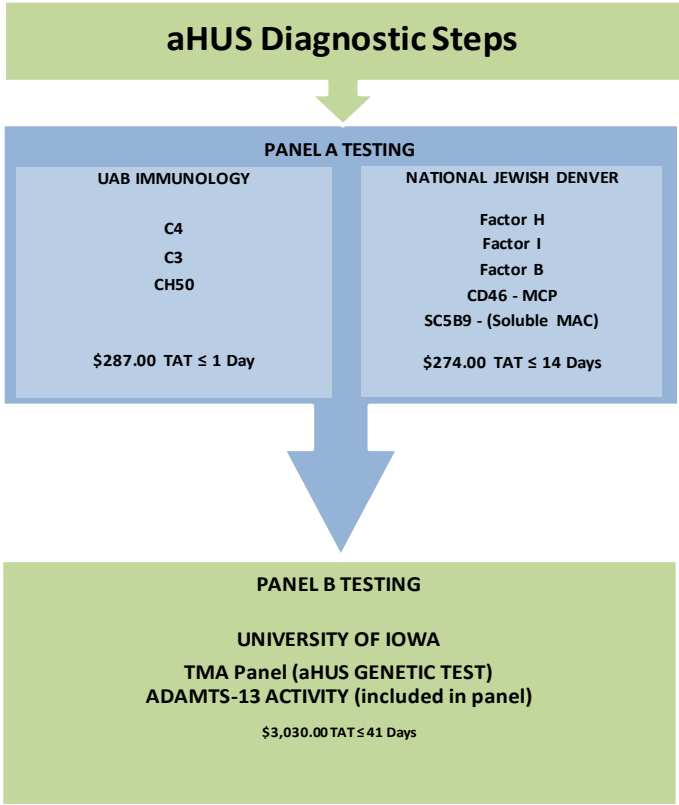
This is should be first and foremost a diagnosis of **exclusion,** and in the setting of **confirmation** of clinical suspicions (b/c the results will not be back for weeks). This test should be sent after testing has demonstrated negative results for infectious/traumatic/metabolic causes of disease. Further the results will take a very long time to come back (>1 week), and as such the clinical team should have plans for how they are going to proceed with treatment in the absence of results.

Further this antibody panel may represent antibodies that are associated with very different underlying processes (ie underlying malignancy versus an auto-immune condition). Contact the clinical team and see not only where this falls in their differential diagnosis (and considering holding testing until other disease entities have been effectively ruled out), but also whether their suspicions are in line with an auto-immune versus paraneoplastic process. If the clinical presentation is suspicious for an underlying malignancy (weight loss/anemia/h/o malignancy/any sort of b-symptoms etc), then the team should start with imaging (brain and body) prior to test submission. Testing may then be submitted for a serum paraneoplastic panel. If the presentation is more characteristic of an autoimmune process (sub-acute/acute presentation, young patient, primarily mood/memory/dementia symptoms etc) then CSF testing should be pursued as there is some evidence in the literature that this may be more sensitive for these antibodies. If the clinical picture is "classic" for auto-immune encephalitis, AND the clinical team has effectively ruled out all other potential etiologies, and is about to proceed with immunomodulatory therapy then the CSF ENCEC may be considered with attending approval.

Atypical Hemolytic Uremic Syndrome (aHUS):

aHUS is a disease characterized by non-immune hemolytic anemia, thrombocytopenia and organ dysfunction such as renal impairment. It is typically caused by uncontrolled activation of the compliment system due to abnormalities in the alternative pathway of complement (e.g., factor H) (Noris and Remuzzi NEJM 361:1676 (2009)). When patients with aHUS have a kidney failure, they require expensive treatment options and are likely to reject the transplanted kidneys. Thus, nephrologists often request complement cascade workup, which is helpful in managing patients as well as assessing the risks of their family members. However the workup can be complex and expensive, since patients often present with a history of extensive transfusions, many complement factors are involved and identifying sequence variants of complement components is important. Following discussions with nephrologists and Dr. S. Harada in Molecular Diagnostics Laboratory, we have developed the following diagnostic pathway in 2014. Also note that aHUS can resemble TTP, which is caused by decreased ADAMTS13 activity.

However, it is becoming increasing common for patients with aHUS to have complement levels within normal limits and still have complement mutations seen on molecular testing. Therefore, if a nephrologist would like the genetic test sent out – the go ahead with the approval, but also make sure the local C3/C4/CH50 is ordered (before any apheresis or therapeutic monoclonal antibody treatment). The test is sent to the University of Iowa and is called the Genetic Renal Panel. It screens 11 genes (*CFH, CFI, MCP, CFB, CFHR5, C3, THBD, ADAMTS13, DGKE, MMACHC* and *PLG)* - *https://morl.lab.uiowa.edu/kidney-disease* . If the ADAMTS13 level is requested, it should be sent separately to the Blood Center of Wisconsin.


aHUS Diagnostic Steps
PANEL A TESTING
UAB IMMUNOLOGY
C4
C3
CH50
$287.00 TAT ≤ 1 Day
NATIONAL JEWISH DENVER
Factor H
Factor I
Factor B
CD46 - MCP
SC5B9 - (Soluble MAC)
$274.00 TAT ≤ 14 Days
PANEL B TESTING
UNIVERSITY OF IOWA
TMA Panel (aHUS GENETIC TEST)
ADAMTS-13 ACTIVITY (included in panel)
$3,030.00 TAT ≤ 41 Days

GLOSSARY OF IMMUNO-ASSAYS:

Soluble Antigen-Antibody Reaction Assays:

***Immunodiffusion* (ID),** also called Double diffusion (DD) or the *Ouchterlony* technique, is the classical procedure used to detect the presence of antibodies and determine their specificity by visualization of "lines of identity" (precipitin lines). These precipitin lines (precipitated antigen-antibody complexes) form where the binding concentrations of antigen and antibody are equivalent. Patient serum diffuses from one well through the gel and reacts with a known specific antigen (or antibody), which diffuses through the gel from a second well. DD is strictly qualitative, although the density of the precipitin line and the distance of the line from the sample well may give some indication of the antibody concentration.

***Radial immunodiffusion* (RID)** is a quantitative variation of the Ouchterlony technique (immunodiffusion) in which the agar gel contains evenly distributed antigen (or antibody) and its counterpart from the test sample diffuses into the gel from a single well resulting in a circular precipitin line around the sample well. The diameter of the precipitin ring is proportional to the concentration of the antibody (or antigen) present in the test sample. By comparing the diameter of the test specimen precipitin ring to known standards, a relatively insensitive estimation of the concentration of specific antibody or antigen can be achieved.

***Counterimmunoelectrophoresis* (CIE)** is a procedure in which oppositely charged antigen and antibody are propelled toward each other by an electrical field. This reduces the time necessary for visualization of the antigen-antibody reaction from 18-24 hours in ID to less than one hour and also substantially increases the sensitivity of the analysis.

***Immunoelectrophoresis* (IEP)** is a two-step procedure, which first involves the electrophoretic separation of proteins, followed by the linear diffusion of antibodies into the electrophoretic gel from a trough, which extends through the length of the gel adjacent to the electrophoretic path. The antigen-antibody reactions procedure precipitin arcs at positions where equivalence occurs. Although quantitation is subjective, an experienced eye can determine not only the presence of the antigen but, through visual comparison to normal control sera, may discriminate relative increases or decreases of antigen by gauging the length and density of the precipitin arcs at positions established for specific antigens using known standards.

***Immunofixation* (IFIX)** is a powerful enhancement of immunoelectrophoresis in which a series of post-electrophoretic gel slabs are layered with cellulose-acetate gels saturated with specific antibodies. The resulting antigen-antibody complexes fixed on the second gel may then be stained, allowing sensitive and specific qualitative visual identification of paraproteins by electrophoretic position.

Direct agglutination (DA) is a general term for techniques, which use the agglutination (macroscopic clumping) of particulate reagents as an indicator of the presence of an antigen-antibody reaction. Examples (HA, LA and CoA) follow.

Hemagglutination (HA) is a technique for detecting specific antibodies which, when present, will cause antigen-coated reagent erythrocytes to agglutinate. Crude quantitation of the antibodies can be achieved by performing a serial dilution of the patient serum and noting the highest dilution (titer) at which agglutination is still present.

Latex agglutination (LA), also known as latex particle agglutination, for detection of antibodies is identical to HA in principle, but the substitution of antigen-coated latex particles for erythrocytes results in improved sensitivity and reagent longevity.

Alternatively, antibodies can be absorbed to the latex particles by binding to the Fc region of antibodies, leaving the Fab region free to interact with antigens present in the applied specimens. This phenomenon has made LA a popular technique for detecting antigens as well.

Coagglutination (CoA) is similar to the LA technique for detecting antigen (described above). The assay involves *Staphylococcus aureus* which expresses protein A that can bind the Fc region of most IgG isotype antibodies leaving the Fab region free to interact with antigens present in the applied specimens. The visible agglutination of the *S. aureus* particles indicates the antigen-antibody reaction.

Hemagglutination inhibition (HI), also abbreviated HAI, is a variation of the HA technique. Some viral antigens, when coated on erythrocytes, spontaneously cause agglutination in the absence of antibody. In these situations, the specific antigen-antibody reaction actually prevents the agglutination of reagent RBCs. HAI cannot differentiate between isotypes of specific antibodies (IgG, IgA or IgM) although positive HAI analysis of specimens treated with Staphylococcus aureus Protein A (discussed above under CoA) to remove the IgG isotype antibodies has been used to imply the presence of specific IgM antibodies to the specific viral antigen. The crude quantitation of the specific antibodies is possible using serial dilution (titer).

Nephelometry (NEPH) is used to quantitate antigen by analyzing increases in light scatter with laser light. The interaction of specific antibodies in the reagent with the antigen from the sample results in the formation of antigen-antibody complexes, which scatter light. Most modern nephelometers compare the rate of formation of antigen-antibody complexes (determined by computer analysis of laser light scatter data) to that of known antigenic standards in order to measure precisely the protein antigens (some of which are actually immunoglobulins) present in moderate

concentrations. Immune complexes can also reduce transmission of light. When the light transmission is measured, the assay method is called turbidimetry.

Complement fixation (CF) is an exacting, complex yet sensitive procedure that detects the presence of a specific antigen-antibody reaction by causing the in vitro activation of complement via the classical pathway. If complement is not activated (i.e., not fixed), lysis of the pre-antibody-coated reagent erythrocytes (e.g., sheep erythrocytes) occurs. Again, crude quantitation of antibodies is possible by determining the highest dilution (titer) at which lysis does not occur. The differentiation of specific antibody isotype is not possible.

Neutralization (Nt) is similar to complement fixation but is used only in certain pathogenic situations where the antibody being measured is directed against a hemolysin (a bacterial toxin capable of directly lysing erythrocytes). In these situations, the hemolysin and reagent erythrocytes are added, and if the antibody to the hemolysin is present, the lysis of RBCs will not occur. As in CF, crude quantitation is afforded by serial dilution which may be quantitatively compared to established standard material dilutions.

Immunohistochemical Assays:

Fluorescent antibody assay (FA) is a general term for procedures which utilize the visual detection of fluorescent dyes coupled (conjugated) to antibodies which react with the antigen when present using fluorescent microscopy. FA allows a competent technologist to identify visually the site of the antigen-antibody reaction thereby rendering specific specificity. Variations are further explained below (DFA, IFA, ACIF, ABIF, and Micro-IF).

Direct fluorescent antibody assay (DFA) is the straightforward detection of antigens using fluorescently labeled antigen-specific antibody. Because detection of the antigen in a substrate of patient

sample (cellular smear, fluid or patient-inoculated culture medium) is the goal, DFA is seldom quantitative.

Indirect fluorescent antibody assay (IFA) is used to detect antibodies to specific antigenic material in the substrate using fluorescent microscopy. Using fluorescently conjugated antibodies specific for a particular isotype of antibody, it is possible to distinguish IgG, IgA and IgM isotypes of specific antibodies using IFA. This sensitive technique is highly effective in well-trained hands and recent developments in the establishment of internationally recognized standard materials have led to the accurate quantitation of antibody concentrations through endpoint titration (the highest serial dilution of specimen at which specific fluorescence remains) and through measuring visual intensity of fluorescence compared to known reference standard material.

Micro-immunofluorescence assay (Micro-IFA) is really multiple IFA. Several different substrates are arranged in specific locations on a single microscope slide well allowing a rapid, simultaneous IFA on each substrate. This is commonly used to detect ANA.

Anticomplement immunofluorescence (ACIF) is a technique used to make certain indirect fluorescent antibody techniques more specific and sensitive. Here the fluorescent dye is conjugated to antibody directed at complement and then added to a complement-fixing complex of antigen and patient antibody.

Avidin-biotin immunofluorescence (ABIF) holds promise for more sensitive and specific amplification of indirect fluorescent antibody procedures. Antibody to the patient's specific antibodies is labeled with biotin, a compound capable of specifically binding avidin in high concentrations. Fluorescently labeled avidin is then added and fluorescent microscopy is used to detect the presence of the complexes.

Immunoperoxidase (IP) assays are analogous to IFA in that antibody presence is identified on antigenic substrates visually. However, in the indirect IP instead of fluorescent dye-antibody conjugates, enzyme-antibody conjugates (principally peroxidase enzymes) are reacted with their corresponding substrates to produce a product which can be seen with a light microscope, eliminating the requirement for costly fluorescent microscopic equipment.

Immunocytochemical assay (ICA) involves the computerized assessment of microscopic fields following DFA, IFA or indirect or direct IP analysis of biopsy tissue from the patient. In addition to improved specificity with the removal of operator subjectivity, the quantifiability of results through computer data analysis of color, intensity and concentration has only begun to be realized.

Various general Immunoassays:

Radioimmunoassay (RIA) uses fixed-dose, low-level, radioactive-isotope-labeled antigen ("tracer") to compete with unlabeled antigen from the patient specimen for a fixed number of antibody binding sites. Traditional RIA is done with specific antibodies in liquid solution. Solid-phase RIA involves the use of antibody bound to solid support (e.g. tubes, glass beads or plastic fins). The amount of antigen in the specimen is determined by comparing the bound radioactivity with a standard curve.

Immunoradiometric assay (IRMA) uses low-level radioactively labeled specific antibody to quantitate low concentration compounds. In IRMA, a first antibody is presented on solid-phase (coated on tubes or beads). After binding the antigen present in the sample, a second radioactively labeled antibody is added. Radioactivity remaining after washing the solid phase is proportional to the concentration of antigen present in the sample and is quantitated by comparison to a standard curve.

Radioallergosorbent test (RAST) is the name given to an *in vitro* technique which detects the presence of IgE (and IgG) antibodies to allergens, proteins which may give rise to hypersensitivity reactions seen in allergies. Allergens are coated on a complex carbohydrate matrix called a sorbent. Antibodies specific for the allergen being tested bind to the allergen and, if present, are detected by a low-level radioactively labeled antibody to either human IgE or IgG, depending on the type of isotype being tested.

Chemiluminescence assays (CIA), including a subcategory using bioiuminescence (biologically derived chemiluminescence agents), use the generation of light from oxidative chemical reactions as an indicator of the quantity of unbound luminescently labeled antigen. This allows quantitation of unlabeled antigen from patient specimens in a variety of homogeneous (single phase) or heterogeneous (multiple phase) immunoassay techniques.

Enzyme immunoassay (EIA) is the general term for many different assays based on antigen and antibody reactions. These tests use color-changed products of enzyme-substrate interaction (or inhibition) to measure the antigen-antibody reaction. Examples of EIA procedures (EMIT, ELISA, MAC, MEIA) follow.

Enzyme-linked immunosorbent assay (ELISA) is a sensitive, heterogenous (multiple phase) analytical technique for quantitation of antigen or antibody in which enzyme-labeled antibody or antigen is bound to a solid support (e.g. tubes, beads, microtiter plate wells, plastic tines or fins). After addition of patient specimen and substrate, antigen, antibody or complex are detected by a color change indicating the presence of the product of an enzyme-substrate reaction. Direct ELISA (or inhibition ELISA) is a technique for measuring antigen using competition for antibody binding sites between enzyme-labeled antigen and patient antigen. Indirect ELISA (or enzyme immunometric assay or sandwich

assay) measures antibody concentrations using bound antigen to interact with specimen antibodies. Enzyme-labeled reagent antibodies can be isotype-specific (i.e., capable of determining the presence of IgG, IgA, IgM or IgE classes which react with the antigen of interest). The specificity of indirect ELISA assays for IgM isotypes in come infectious diseases is limited by false-positive results due to IgM rheumatoid factor in the presence of IgG-specific antibodies.

IgM antibody capture ELISA (MAC ELISA) has been developed to impart significant improvement in assay specificity to indirect ELISA procedures for IgM isotype antibodies. Solid-phase support *(usually microtiter plate wells) is coated with anti-*human IgM antibodies capable of binding all IgM isotype antibodies present in the specimen. Test sample is then added, followed by enzyme-labeled antigen-specific antibodies. If IgM antibodies specific for the antigen in question are present, the "sandwich" complex will result in enzymatic color-change proportional to the concentration of IgM-specific antibody present. This technique appears to be the method of choice in many highly specific and more sensitive assays for IgM infectious disease antibodies.

Micro particle enzyme immunoassay (MEIA) is a technique in which the solid-phase support consists of very small micro particles in liquid suspension. Specific reagent antibodies are covalently bound to the micro particles. Antigen, if present, is then "sandwiched" between bound antibodies and antigen-specific, enzyme-labeled antibodies. Antigen-antibody complexes are detected and quantitated by analysis of fluorescence from the enzyme-substrate interaction.

Radioimmunoprecipitation assay (RIPA) is the term used to describe the qualitative assay used as a confirmatory procedure for some antibodies to viral antigens. Viral-infected cell cultures are

radioactively labeled and lysed to yield radiolabeled antigen fragments. Specific antibodies, if present, will bind these antigen fragments and the resulting antigen-antibody complexes are precipitated using protein A, boiled to free the immune complexes which are then separated by electrophoresis. The pattern of antigenic moieties to which antibodies are present may then be detected using autoradiography (the exposure of sensitive X-ray film by the radioactive emissions of the bound, labeled antigens). Comparison to labeled molecular weight standards electrophoresed in the same run allows determination of the molecular weight "bands" of antigen to which antibodies are present.

Fluorescence polarization immunoassay (FPIA) is a technique which takes advantage of the increased polarization (non-random propagation of emission) of fluorescent light emissions when a fluorescently labeled antigen is bound by reagent antibody. The higher the concentration of unlabeled patient antigen present in the test mixture, the less bound fluorescent antigen is present, and consequently, the lower the polarization of the fluorescent light emission. Standard calibration yields quantitative results.

Enzyme multiplied immunoassay technique (EMIT) is a homogeneous (single phase) EIA procedure in which the antigen being measured competes for a limited number of antibody binding sites with enzyme labeled antigen. The reagent antibody has the ability to block enzymatic activity when bound with the reagent enzyme-antigen complex preventing its formation of product in the presence of substrate. The free antigen-enzyme complexes resulting from competition with measured antigen in the sample forms color-change products proportional to the concentration of antigen present in the specimen.

Atypical HUS

-see p. 92-

Free Light Chains

These tests are used as an aid in the diagnosis and monitoring of multiple myeloma and related disorders. Serum free light-chain assays are superior over the urine levels, especially in disorders where there are low levels produced (like non-secretory multiple myeloma or amyloidosis), because these light chains are reabsorbed in the kidney. Patients with an abnormal free kappa / free lambda (normal range is 0.3 – 1.2) have an increased risk of progression to active myeloma from MGUS. This assay should be done in combination with serum protein electrophoresis and immunofixation. A good reference is: Am J Hematol. 2010, Oct;85(10):787-90. doi: 10.1002/ajh.21815. Serum free light chain analysis. Davids MS, Murali MR, Kuter DJ.

IgA Levels

-see p. 76-80

Paraneoplastic (and other Neuronal) Antibodies -see pages 82-91

QuantiFERON®-TB Gold (QFT-G) test result is Indeterminate. What does this mean? (NOTE – this test is no longer offered at UAB and has been replaced with a sendout test (T-SPOT).

Test kits include a mixture of synthetic peptides representing ESAT-6 and CFP-10 as TB test antigens, phytohemaglutinin (a mitogen used as a positive assay control), and saline (used as a nil sample to measure the background level of IFN-γ). After incubation, the concentration of IFN-γ in the plasma is determined by ELISA using the reagents included in the test kit. The amount of IFN-γ released is determined by subtracting the amount in the

nil from the amount in the ESAT-6, CFP-10, or mitogen-stimulated plasma.

TABLE. Interpretation of QFT-G* results, from IFN-γ† concentrations in test samples

ESAT-6–nil§ or CFP-10–nil¶ or both	Nil	Mitogen–nil**	QFT-G result	Interpretation
≥0.35 IU/mL†† and >50% above nil	Any	Any	Positive	*Mycobacterium tuberculosis* infection likely
<0.35 IU/mL	≤0.7	≥0.5	Negative	*M. tuberculosis* infection unlikely but cannot be excluded, especially when illness is consistent with TB§§ disease and likelihood of progression to TB disease is increased
<0.35 IU/mL	Any	<0.5	Indeterminate	QFT-G results cannot be interpreted as a result of low mitogen response
≤50% above nil	>0.7	Any	Indeterminate	QFT-G results cannot be interpreted as a result of high background response

* QuantiFERON®-TB Gold test.
† Interferon-gamma.
§ The IFN-γ concentration in blood incubated with a mixture of synthetic peptides simulating early secretory antigenic target–6 (ESAT-6) minus the IFN-γ concentration in blood incubated with saline.
¶ The IFN-γ concentration in blood incubated with a mixture of synthetic peptides simulating culture filtrate protein–10 (CFP-10) minus the IFN-γ concentration in blood incubated with saline.
** IFN-γ concentration in blood incubated with mitogen minus the IFN-γ concentration in blood incubated with saline.
†† International units per mL.
§§ Tuberculosis.

In the lab, quantitative values from the TB antigen containing tubes are compared to negative ("nil tube") and positive ("mitogen tube") controls, which determine the validity of the test. If the negative control has levels of IFN-γ that are inappropriately high, the test is considered a "high nil" indeterminate. Likewise, a "low mitogen" indeterminate result can occur due to an inappropriately low IFN-γ response to mitogen in the positive control. This may indicate the specimen was mishandled (tubes not maintained at room temperature during transport, tubes over or under filled, delivery to lab delayed beyond 16 hours etc.) or that the patient's lymphocytes were not reactive enough at baseline for their

measured responses against TB antigens (TB Ag) to be interpreted (this situation is more common in settings of immunosuppression).

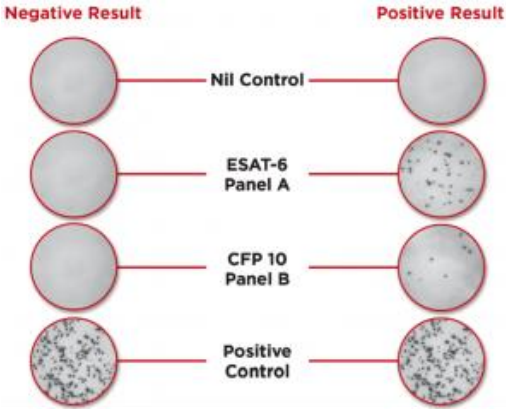
An indeterminate QFT-G result does not provide useful information regarding the likelihood of *M. tuberculosis* infection. The optimal follow-up of persons with indeterminate QFT-G results has not been determined. The options are to repeat QFT-G with a newly obtained blood specimen, administer a TST (Tb skin test), or a sendout T-Spot test which is also an interferon release assay but uses a known number of mononuclear cells, or do stop testing. For persons with an increased likelihood of *M. tuberculosis* infection who have an indeterminate QFT-G result, administration of a second test, either QFT-G or T-Spot, might be prudent. In settings conducting serial testing, the potential for TST to cause boosting and the need for two-step testing should be considered. For persons who are unlikely to have *M. tuberculosis* infection, no further tests are necessary after an indeterminate QFT-G result. Laboratories should report the reason that the QFT-G result was indeterminate (e.g., high background levels of IFN-g in the nil sample or inadequate response to mitogen).

For more information:
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a4.htm#tab

T-SPOT.TB Test

This is also an interferon-gamma release assay, but in this test, the Interferon-gamma is captured by an antibody on a membrane and then detected by a secondary antibody as a "spot". T-cells sensitized to TB infection should produce interferon-gamma that will be secreted and then captured. This type of testing is known as an ELISPOT. The results are interpreted by subtracting the spont cunt in the negative (NIL) control from the spot count in Panels A (ESAT-6) and B (CFP 10). The results are read as

(Positive >8 spots total; Negative <4 spots total;
Borderline = 5, 6, or 7 spots; invalid).



Thyroid Stimulating Immunoglobulins (TSI)
This is a second-line test for autoimmune thyroid disease, used when diagnosing clinically suspected Graves' disease or in helping with the differential diagnosis of the etiology of thyrotoxicosis (especially if thyroid radioisotope scans are indeterminate or contraindicated). They can also be called long-acting-thyroid-stimulators (LATS) and are autoantibodies that bind and activate the thyroid stimulating hormone receptor (TSHR). These antibodies are IgG antibodies and can cross the placental barrier, causing neonatal thyrotoxicosis.

Serology

Dengue Fever IgM/IgG
Dengue fever (DV) is the most prevalent mosquito-borne viral disease in humans. The indications for testing are a flu-like illness with exanthem in endemic area or a patient with appropriate travel history (tropics or subtropics).
For laboratory criteria for diagnosis see:
http://www.arupconsult.com/Topics/DengueFever.html#tabs=0
IgM can be used if one has two samples (one <5 days from onset (acute) and one >5 days from onset (convalescent)). In this case, one is looking for seroconversion. Also need paired samples for IgG looking for >4-fold rise in titer.

Echovirus Antibodies
Echoviruses are non-polio enteroviruses with infections that commonly peak in July – October. The most common types are: Echoviruses 1-9, 11-27 and 29-31.
- Hand-foot-mouth disease (echovirus 4-6)
- Aseptic meningitis (echovirus 6, 7, 9, 10)

Testing Recommendations:
Nucleic acid amplification testing (eg, RT-PCR)
recommended for CSF and blood specimens
Much more sensitive than culture
Rapid turnaround time aids in clinical management of patient
Culture – respiratory specimens, stool
Up to 1 week required for growth
Serologic antibody titers
Requires acute and convalescent titers

Hepatitis C Antibodies
Initial testing – rule out hepatitis A (HAV) or B (HBV) in acute presentation
Perform HAV antibody IgM, HBV core antibody IgM, HBV surface antigen, and HCV antibody testing

Positive HCV from hepatitis panel – perform HCV RNA viral load.
Undetectable viral load – infected but recovered or false-positive screen
Viral load test positive – currently infected a second test in 6 months will differentiated acute vs. chronic HCV.

Lyme Disease Antibodies
For Lyme disease, the first thing is patient history. Lyme disease's largest endemic areas range from Virginia to Maine and through Minnesota and Wisconsin. Testing for Lyme disease should only be ordered if a STRONG clinical suspicion exists. Alabama has reported 17 cases of Lyme disease in 2014 and 25 cases in 2015. There are currently 6 counties in Alabama that are considered to be endemic – Calhoun, Chambers, Jefferson, Mobile, Shelby, and Tuscaloosa. Furthermore, the CSF is not the first place to look for Lyme disease. Look for antibodies in the serum first (see Lyme section), There have not been any well-documented cases of seronegative Lyme disease with demonstrable *B. burgdorferi* DNA in the CSF. Also, the lay public is often familiar with IGeneX, Inc. due to its heavy promotion as a Lyme disease reference laboratory but it is NOT an approved laboratory for Lyme testing.
-see pages 69-71, p. 54 for additional information.

Syphilis Testing (TPPA, FTA, VDRL, RPR)
-see pages 58-61

Zika virus testing in pregnant women: new guidelines for interpreting serologic results (May 17, 2017)

Introduction
The Centers for Disease Control and Prevention (CDC) recently issued new guidance to medical providers on how to interpret the results of Zika IgM serologic tests. Recent studies have indicated that Zika IgM antibodies may persist for prolonged periods in some infected persons, making it difficult to establish whether recent infection has occurred. These findings have the greatest implications for the management of pregnant women who may have been infected with Zika virus before becoming pregnant, such as those women who reside in or who have frequently traveled to areas of local transmission.

Background & Previous Testing Recommendations
Until the release of these most recent guidelines, prior recommendations for Zika testing in pregnant women stated that all women with possible exposure to Zika virus infection (because of travel to areas of active transmission, or from sexual contact with a partner who traveled) should be evaluated for possible infection, whether or not symptoms of Zika virus infection are present. The choice of testing depends on the interval since the last potential exposure to Zika:

- For pregnant women whose last potential exposure to Zika virus was within the preceding 2 weeks, nucleic acid testing (NAT) of serum and urine for Zika virus, such as rRT-PCR testing, should be done.
- For pregnant women whose last potential exposure occurred between 2-12 weeks ago, testing for Zika IgM antibodies is recommended.
- For those pregnant women who present for care more than 12 weeks after the last potential exposure, testing for Zika IgM antibodies may be considered. If fetal abnormalities are present, NAT of the mother's serum and urine testing for Zika virus may be done; however,

negative Zika IgM antibody results or negative NAT results do not rule out Zika infection.

Updated Guidance

Because Zika IgM antibodies have been found to persist in some persons beyond 12 weeks after infection, it is now recognized that the detection of Zika IgM antibodies in a pregnant woman might represent an infection that occurred before conception. Updated recommendations now include the following:

- Recommendations for symptomatic pregnant women remain unchanged.
- Screen all pregnant women for risk of Zika exposure and for any symptoms of Zika. Promptly test pregnant women with NAT testing if they become symptomatic during their pregnancy or if a sexual partner tests positive for Zika virus infection.
- For those asymptomatic pregnant women who reside in or frequently travel to areas with active Zika transmission, or who have sexual partners who test positive for Zika because of symptoms of Zika infection, testing for Zika nucleic acid by NAT should be performed at least once per trimester, in addition to the Zika IgM antibody testing that was previously recommended (unless testing has been previously positive). However, if a symptomatic pregnant woman is IgM positive and NAT negative, and lived in or traveled to an area with a posted CDC Zika Travel Notice, healthcare providers should recognize that the positive IgM result does not necessarily indicate recent infection.
- Consider NAT testing of amniocentesis specimens from women with potential Zika exposure if amniocentesis is performed for other reasons.

- For non-pregnant women who reside in or frequently travel to areas with active Zika transmission, consider IgM testing to determine baseline Zika virus IgM levels as part of preconception counseling.

The current list of countries with posted Zika Travel Notices can be found here: https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika.

Sources

https://emergency.cdc.gov/han/han00402.asp
https://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm

NOTES: