

Diagnos-Techs™
Clinical & Research Laboratory
Quarterly Newsletter

Articles in this edition

- 1 *Improvements in Microbiology Reporting at Diagnos-Techs™*
- 3 *The Therapy Corner — Toxoplasma Gondii*
- 6 *Diagnos-Techs™ Introduces — John J. White, MD CM and Jeremy Celver, PhD*
- 7 *Shelf Life and Shipping Concerns for Test Kits*

Conferences & Tradeshows



If you are attending one of the events listed below, please visit our booth. We look forward to meeting you in person!

A4M at the Gaylord Palms in Orlando, FL on April 11-13th, 2013. **Booth #822**

Digestive Diseases Week (DDW) at the Orange County Convention Center in Orlando, FL on May 19-21st, 2013.

Booth #1700

Improvements in Microbiology Reporting at Diagnos-Techs™

John J. White, MD CM, Kelly Johnson, BS, and John Reinhard, PhD



The previous issue of ChronoBiology Letter reported the acquisition by Diagnos-Techs™ of the MALDI-TOF Mass Spectrometer for the rapid and precise detection of over 4000 microorganisms, both bacterial and fungal, from the stool specimen submitted as part of our Gastrointestinal Health Panels. In association with the Vitek 2 system, bacterial sensitivity to antibiotics can be established for pathologic organisms identified. These constitute a distinct improvement over our prior diagnostic capability.

Hitherto, we relied on well established commercial laboratories for diagnosis using traditional culture and identification techniques. These techniques involved meaningful time delays, and, sometimes, incomplete diagnoses, especially of the less common organisms. The improvements incorporated into the microbiology department of our laboratory correct these deficits and provide many distinct benefits.

First, all of the handling and identification remain at Diagnos-Techs. As a result, identification of organisms is far more complete since it relies on matrix assisted laser desorption ionization, time of flight (MALDI-TOF) mass spectrometry. It preserves the molecular structure and is not affected by the vagaries of culture techniques. Moreover, organisms can be accurately recognized very rapidly. All this can be accomplished generally within two days of receiving the stool specimen. Identification of pathogenic organisms, which can be transmissible and, frequently, are responsible for dangerous outbreaks of disease, can be reported promptly.

Some background is warranted. There has been an increase of scientific interest regarding the human microbiome, that consortium of microorganisms in the human gut, with which we live in harmony or upset. These robust clusters (enterotypes) are not “nation or continent” specific, per se and



Continued on page 2.

Improvements in Microbiology continued from front cover.

their variation generally is stratified and not continuous. These well balanced host microbiotic symbiotic states can respond differently to diet, drug intake, and disease-producing harmful microbes (i.e., pathogens).

The MALDI-TOF and Vitek 2 technologies installed at Diagnos-Techs not only provide accurate and rapid analysis, but they can identify specifically those organisms that are pathogenic. Fortunately, the vast majority of organisms found are part of the regular microbiome of the GI tract. Antibiotic therapy seldom is warranted, even if there is an imbalance. Attention to gentle bowel cleansing and probiotic administration can insure a proper microbiomal population and balance.

These organisms can be thought of as “commensal”, a relationship in which organisms live in close attachment or partnership, and in which none are parasitic or harmful to the other or to the host. A “pathogenic” organism, on the other hand, is one that causes disease. Fortunately, pathogenic organisms are few and far between. Generally, pathogens need to be identified swiftly and often must be reported to State Departments of Health.

The ‘state of the art’ diagnostic equipment in the microbiology department at Diagnos-Techs constitutes the finest such technology available. When pathogenic organisms are identified, appropriate confirmatory diagnosis and antibiotic sensitivities are established using the Vitek 2 equipment. All this information is reported both to the clinician and the appropriate Department of Health, if necessary, within the required time frame. In the

first few months of this new analytic program, several cases of Salmonella and Yersinia infections have been identified.

In view of this new capability for rapid and precise diagnoses, Diagnos-Techs now will report microbiologic results separately from and more promptly than the remainder of the GI Panel results. The assays comprising the latter require more time to complete and will be reported in the usual time frame.

As well, in view of the considerably widened spectrum of conditions which can be assessed, not only will significant positive bacterial growth be reported, but important negative analyses for meaningful toxins,

antigens and bacterial growth that have been assayed. An example of the new microbiology report from Diagnos-Techs is presented in Figure 1.

Overall, in view of these significant improvements, Diagnos-Techs should be considered as a provider’s laboratory of first choice for the microbiologic analysis of stool samples. Our reference lab level testing eliminates the need for time-wasting resubmission of potential pathogenic specimens to a reference laboratory; plus, we can provide the most up-to-date, complete, accurate, and prompt findings for patients at the time of initial specimen submission.

Code	Test Name	Result / Notes	Reference Values/Key
CS1	Stool Cu. Fungi, Isol. & I.D.	+3Saprophytic fungi	+1=Trace +2=Light +3=Moderate +4=Abundant +5=Confluent
GP2	Ova & Parasites, x3 (Stool)	The following parasites were detected: Blastocystis hominis - many Dientamoeba fragilis - rare Endolimax nana - moderate	
GP3	Bacterial Stool Cu.	***Potential Pathogen Isolated*** Yersinia enterocolitica - Moderate growth Ampicillin Resistant Cefazolin Resistant Ceftazidime Susceptible Gentamicin Susceptible Ciprofloxacin Susceptible Levofloxacin Susceptible Trimeth/Sulfa Susceptible ***Other findings Heavy growth mixed Gram negative rods/flora. Moderate growth mixed Gram positive rods/flora. No Salmonella, Shigella, or E coli O157 isolated No Vibrio or Aeromonas isolated No Proteus or Pseudomonas isolated	<i>Expected Findings:</i> - Moderate to heavy growth of mixed Gram (+) & (-) flora - No pathogens should be detected.
GP3CA	Campylobacter Antigen	Negative	Normal: Negative
GP3ST	Shiga Toxins 1 & 2 (Stool)	Negative	Normal: Negative

Figure 1.

Abridged results from new microbiology report. Note the presence of three parasites by microscopic examination (O&P), isolation of the potential pathogen (Yersinia enterocolitis, antibiotic sensitivity, and pertinent negative findings).



The Therapy Corner

Toxoplasma Gondii

Brandy Webb, ND

Introduction

Toxoplasma gondii is a microscopic parasite that, upon ingestion, may replicate and develop into tissue cysts that reside within the body for years. About one in ten individuals is infected with toxoplasma in the United States, although most are asymptomatic.¹ Human toxoplasmosis can manifest in a number of forms: acute infection, latent infection, or reactivation. The majority of infected persons fall into the latent category, characterized by presence of T. gondii tissue cysts and certain positive laboratory findings but without symptomatology. In this phase cysts are contained by immunologic barriers that prevent systemic spread. In immunocompetent persons, treatment may be required during the initial acute infection, if symptoms are severe, or at times of reactivation of latent parasitic organisms.

Transmission of Toxoplasma gondii in humans usually occurs from eating undercooked meat from animals that were infected (usually pork or lamb) or through exposure to feces from infected cats (including from litter boxes or contaminated soil or water). In addition, vertical transmission from mother to fetus can occur in cases where the mother is newly infected during pregnancy.

Up to ninety percent of acute Toxoplasma gondii infections that affect individuals without preexisting immune deficiencies are completely asymptomatic.² When clinical manifestations do occur during

acute infection, they include flu-like symptoms such as fever, chills, headaches, muscle aches, and sore throat. Lymphadenopathy is another common presentation—occasionally generalized, but more often involving the cervical lymph nodes only. These symptoms typically last several weeks to several months, although some patients may experience them for up to a year.² Most cases of toxoplasmosis among immunocompetent patients will enter the latent phase and remain that way, with no long term sequelae whatsoever. During this phase, the parasite forms cysts that reside mainly in the brain, skeletal muscle, heart muscle, and the eyes. Healthy patients' immune systems prevent the parasites from proliferating and causing greater health problems. If a patient's immunity becomes significantly compromised, the latent parasitic cysts can become reactivated and affect a variety of organ systems. A compromised immunological state may result from HIV/AIDS, organ transplant anti-rejection treatment, or the use of immunosuppressive drugs to treat autoimmune diseases such as Crohn's disease or rheumatoid arthritis. Most commonly, immunocompromised patients with toxoplasmosis suffer encephalitis presenting with headache, confusion, fever, and sometimes seizures.³ Other manifestations of reactivated Toxoplasma gondii involve the lung (presenting with dry cough, shortness of breath, and fever) and the retina (causing eye pain and changes in vision).⁴

Toxoplasma Gondii Testing

Various anti-Toxoplasma gondii immunoglobulins are produced during the course of infection, but simply testing for these may not be sufficient to make a definitive diagnosis of toxoplasma. Clinical presentation and the immune competence of the patient are two major factors that inform the diagnostic approach. Evaluating for T. gondii infection in pregnant woman and neonates generally requires following a complex algorithm of testing at specific times during gestation and the postnatal period. The scope of this article is limited to nonpregnant adults; for a comprehensive discussion on diagnosing and treating pregnant females and neonates, please see "Management of Toxoplasma gondii Infection during Pregnancy" by Montoya and Remington.⁵

When evaluating for acute, primary toxoplasmosis, serologic IgG and IgM testing is a common first step after clinical assessment. If the anti-Toxoplasma gondii IgM titers are negative in immunocompetent individuals, acute infection is almost certainly ruled out. However, IgM titers can remain elevated for months or years after primary infection, even if the infection becomes latent. Elevated IgG titers occur mostly in latent infections and may be present for years. Patients with immune compromise whose latent infections become reactivated typically show positive IgG but negative IgM to T. gondii.⁶ Initial positive findings should be confirmed through additional testing in most cases; the most frequent follow-up tests include avidity, agglutination, or PCR assays.

Continued on page 4.

The Therapy Corner
continued from page 3.

While less common, IgA-based *Toxoplasma gondii* tests are also available. Evidence suggests that positive IgA (including secretory IgA) findings may correlate more with acute infections; numerous researchers, however, have found elevated IgA/SlgA even with latent toxoplasma. This is shown through blood, saliva, stool, and even tears.^{7,8,9} All positive findings, including salivary SlgA to *T. gondii*, must be interpreted in context of the clinical picture. If an acute infection or reactivation is suspected, it is recommended that confirmatory testing be done before initiating treatment.

Conventional Treatments

Deciding whether to treat toxoplasmosis largely hinges on the phase of the infection (acute, latent, or reactivated), if the patient is immunocompromised or pregnant, and the severity of symptoms. Pharmacologic protocols are generally unnecessary in immunocompetent, nonpregnant patients, unless symptoms are severe or last beyond a few weeks. The vast majority of cases will resolve (i.e., transition from acute to latent) without intervention. When drug therapy is needed, pyrimethamine (Daraprim®) is generally given along with another drug such as sulfadiazine, clindamycin (Cleocin®), azithromycin (Zithromax®), or atovaquone (Mepron®).² As a folic acid antagonist, pyrimethamine is an effective antiparasitic agent against *T. gondii*, but this property also necessitates folic acid supplementation (often in the form of leucovorin calcium) in patients taking pyrimethamine. The most common first line therapy consists of:

**Pyrimethamine (Daraprim®)—100mg
PO on day one then 25-50mg QD**

**Sulfadiazine—2-4g PO QD
in four divided doses**

Leucovorin calcium—10-25mg PO QD

Since pyrimethamine antagonizes folic acid, it should be avoided in patients with aplastic anemia. An alternative regimen for these patients or people sensitive to pyrimethamine is a combination of trimethoprim and sulfamethoxazole.² Nitazoxanide (Alinia®) has also been studied as a treatment for toxoplasmosis, although a standard dosing regimen has not been established.¹⁰ Irrespective of the specific regimen chosen, drug therapies typically last between two and four weeks.

Special patient populations require modified drug therapies. HIV/AIDS patients with acute or reactivated toxoplasmosis usually require higher dosing than immunocompetent patients, such as the following:

**Pyrimethamine (Daraprim®)—200mg
PO on day one then 50-75mg QD**

**Sulfadiazine—4-6g PO QD
in four divided doses**

Leucovorin calcium—10-25mg PO QD

Pregnant women are usually treated if they acquire toxoplasmosis while pregnant, and the typical regimen involves the standard pyrimethamine plus sulfadiazine protocol if the infection occurs during the second or third trimester. If the infection occurs during the first trimester, however, spiramycin (4g PO QD in four divided doses) should be prescribed instead, due to the teratogenic nature of pyrimethamine. In the U.S., spiramycin is regulated tightly and can only be acquired by contacting the FDA directly (301-827-2335).^{11,12}

Toxoplasmosis in the newborn should be treated using pyrimethamine and sulfadiazine for the first year of life. When treating *Toxoplasma gondii* in any of these special populations, consider consulting with or referring your patient to an infectious disease specialist.

Natural Treatments

Many natural substances demonstrate anti-parasitic properties, although more studies are needed to prove the efficacy of natural treatments against *Toxoplasma gondii*. The following have shown promising anti-*Toxoplasma gondii* activity, either by acting on the parasite directly or by increasing host immune response:

**Curcuma longa (curcumin)¹³
Astragalus membranaceus¹⁴
Scutellaria baicalensis
(Baikal skullcap)¹⁴
Zingiber officinale (ginger)^{15,16}
Sophora flavescens^{16,17,18}
Artemisia annua¹⁹
Changqing capsule²⁰
L-citrulline or L-arginine²¹**

Most research on natural therapies for toxoplasmosis involves *in vitro* experiments or animal studies; thus, it is impossible to know the full implications for humans *in vivo*. This fact notwithstanding, numerous herbs have exhibited therapeutic potential. Curcumin and ginger are of particular interest since they may be incorporated readily into the diet. Curcumin appears to strengthen the immune response to *Toxoplasma gondii*, whereas ginger shows direct cytotoxic effects against the parasite.^{13,16} Evidence suggests that Baikal skullcap and Astragalus augment the immune response against *T. gondii*, whereas Artemisia and Changqing capsule (a traditional Chinese medicine) inhibit the parasitic

organism directly.^{14,19,20} L-citrulline and L-arginine both enhance *T. gondii* destruction by increasing nitric oxide levels, which enhances the function of activated macrophages.²¹

These natural treatments may be prescribed alone or in conjunction with conventional drug therapies for immunocompetent, nonpregnant adults. For special patient populations (immunocompromised patients, pregnant women, neonates), these should not be considered a substitute for conventional therapy.

Prevention

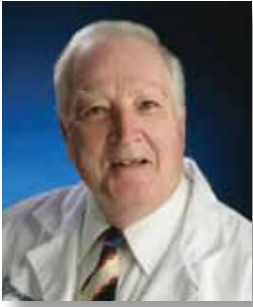
The best way to combat *Toxoplasma gondii* is to prevent infection in the first place. There are several specific actions that one may take to minimize exposure risk. First, avoid eating undercooked meat. The CDC recommends cooking whole cuts of meat to 145°F, ground beef to 160°F, and poultry to 165°F.³ Clean preparation surfaces, cutting boards, and utensils to avoid cross-contamination. Wash all fruits and vegetables thoroughly in case they were grown in soil contaminated with toxoplasma. And finally, avoid exposure to feces of outdoor cats, since the *T. gondii* cysts are readily shed into the feces of cats with primary toxoplasmosis. Wearing gloves while changing litter boxes along with careful hand washing afterward can aid prophylaxis. By adhering to these guidelines, patients can significantly reduce their risk of infection.

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Diagnos-Techs™ Introduces—



Chief Medical Adviser

John J. White, MD CM

After an active and productive academic and clinical career as Professor of Surgery and Pediatrics, Dr. White and his wife retired to Seattle to enjoy the company of their children and grandchildren. Somewhat later, he was recruited to join Diagnos-Techs™ as a medical consultant and educator. He has been appointed now to the post of Chief Medical Adviser and Chair of the Scientific Advisory Board at Diagnos-Techs.

Following college at Fordham University in The Bronx, New York, Dr. White received his MD CM degree from McGill University in Montreal, Quebec. He interned at the University of Michigan, and then spent two years in the U.S. Public Health Service assigned to the U.S. Coast Guard Academy in New London, Connecticut. He returned to the McGill University Teaching Hospitals (Royal Victoria and Montreal Children's Hospitals) where he qualified in both General and Cardio-Vascular and Thoracic Surgery. Pursuing his interest in Pediatric Surgery, he trained further at the Johns Hopkins Hospital in Baltimore, after which he remained on staff there for 10 years.

Dr. White was Professor of Surgery and Pediatrics and Chief of Pediatric Surgery at the Albany Medical College, Albany, NY, Loma Linda University, Loma Linda, CA, and the Mercer School of Medicine, Macon, GA. He has been a member of many prominent surgical societies and authored or co-authored more than 175 peer-reviewed articles, book chapters, and letters; he was co-editor of three text books.

In his new position at Diagnos-Techs, Dr. White will be responsible for the medical application and promulgation of the ever-widening panoply of tests and panels offered by Diagnos-Techs, and for overall external relations with clinicians and agencies.



Assistant Laboratory Director Jeremy Celver, PhD

Dr. Celver received his BS degree in Molecular Cell Biology from the University of Washington. He then earned his PhD degree from the Department of Pharmacology at the U.W., following which he spent a post-doctoral year there. Subsequently, as a member of the Medical Service Corps of the U.S. Army, he was assigned as the Chief of Cell Biology, Department of Clinical Investigation,

and the Supervisor of Core Laboratory (Chemistry and Hematology), Department of Pathology, at Madigan Army Medical Center. In these positions, Dr. Celver mentored research scientists and helped support and guide graduate medical educators in clinical and basic research areas. He anchored the Molecular Biology course for research students and fellows, while researching Botulinum neurotoxin counter measures. He coordinated the IRB-approved study of maternal plasma and was a member of their Institutional Review Board. At the Madigan Laboratories he also supervised section heads overseeing more than 30 technicians and ensured clinical laboratory compliance with all accrediting agencies.

For the past five years, Dr. Celver was the Scientific Director and Principal Investigator at Kovogen, LLC, and coordinated research with the University of Rhode Island and the University of Massachusetts. He was Principal Investigator for research aimed at developing novel probes for imaging brain injuries, and he was concurrently an Adjunct Research Professor at the University of Rhode Island, Biomedical and Pharmaceutical Sciences, originating and directing research to better understand the role of dopamine receptors in schizophrenia and Parkinson's disease. In this capacity, he mentored postdoctoral, undergraduate, and graduate student researchers. Additionally, he has authored 19 peer-reviewed published articles.

At Diagnos-Techs™, Dr. Celver is the Assistant Laboratory Director, helping direct the clinical laboratory, and participating in the active research efforts ongoing in the laboratory.

Shelf Life and Shipping Concerns for Test Kits

John J. White, MD CM, and
Ning Cegielska, MS, CMT



For the most part, the test kits supplied by Diagnos-Techs™, have a long shelf life provided that the integrity of the vials included in the test kit have not been compromised (i.e., they have not been opened and possibly contaminated). Certain vials, however, contain preservatives that must be of a proper concentration to insure that the test results are accurate. Specifically, these are the two “B” stool collection vials (brown top) and the “FL” vial (green top), which is used for the FSH and LH assays (included in all expanded hormone panels). Viability of the “B” vials is limited to one year from the date it was prepared. The expiration date can be found on the test kit box; all “B” vials must be replaced before use if beyond this date.

Once the vials are used, (i.e., receive a specimen such as stool or saliva from a patient), certain time restrictions apply. Some test vials must arrive at the laboratory within a relatively short time span; for others, there is little concern for shipping time.

The two “B” stool vials are used for all stool antigen assessment, inflammatory marker measurements, and microscopic evaluation for ova and parasites (O&P). Their preservative lasts seven days from collection to assay, necessitating a constrained time for shipping and testing. The “A” stool

vial contains saline and is used for the stool and yeast cultures. The “A” stool vial also must be received within seven days to prevent overgrowth of fungi and bacteria. In view of this short window, when doing the GI panel, the “A” sample should be collected last (just prior to shipping). If the patient is unable to ship immediately, the sample may be refrigerated (NOT FROZEN) temporarily.

The “FL” vial contains a preservative, which insures accurate FSH and LH assessment. Of these two, the FSH is the most critical. This vial must be received within 14 days of collection to provide accurate assay. Freezing these vials After Collection (e.g., during the cycling Female Hormone Panel) circumvents this problem. If unable to ship immediately, these vials can be frozen temporarily.

Diagnos-Techs prepays all return shipping of the test kits from patients to the laboratory under an arrangement with United Parcel Service (UPS). UPS will deliver the test kits within 3-4 days of receipt. On occasion, a delay may occur with an office/patient delivery to a UPS store or UPS pickup, or a weekend may intervene. In such cases, temporary refrigeration or freezing, as appropriate, may be required.

Prior to using any of these collection vials, visually check for preservative. If unsure, or if the Gastrointestinal Panel kit has spent one or more years on a shelf, it is wise to order a replacement. As well, Requisition Forms, Patient Instruction sheets or Shipping Instructions may have changed in the interim.

Attention to these details can assure that a test panel will be analyzed properly on first submission to the laboratory and can prevent the necessity for specimen resubmission.



Diagnos-Techs™ free webinars are filled with insight, practical advice, and real-world case studies to help healthcare professionals maximize patient health and well-being with our accurate, non-invasive tests. Presented by members of our medical staff, our monthly one-hour presentations are ideal for busy practitioners who want to get up to speed quickly on female hormone evaluation, adrenal health, gastrointestinal dysfunction, male estrogen dominance, infertility, and other frequently requested topics.

Upcoming Free Webinars:


April 11th, 2013 –

Chronic Pain and the Stress Response

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Please visit www.diagnostechs.com for more information about our free webinars.



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- **US Domestic:** Deliver completed test kit box to any **UPS** location. **www.ups.com/dropoff** Return shipping to Diagnos-Techs™ is **PRE-PAID**. Kits will arrive within three business days of shipment.



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