ChronoBiology LETTER

DiagnosTechs™ THE LEADING LAB IN SALIVA TESTING SINCE 1989

Winter 2013 — 13th Edition

Exciting Developments at Diagnos-Techs™

Diagnos-Techs™ hosts Dr. François Peyron, venerable parasitologist and renowned toxoplasmosis expert

Dr. François Peyron and Dr. Maroun El Khoury

This past fall, Diagnos-Techs™ invited Dr. François Peyron to present his current research findings on saliva-based Toxoplasma gondii immunoglobulin detection. Dr. Peyron serves as the head of Parasitology and Tropical Medicine at the Hôpital de la Croix Rousse in Lyon, France, and is heavily involved in clinical research, including investigating alternatives

Continued on back cover.

Enhanced Gastrointestinal Laboratory Testing through Combined Stool and Salivary Analysis

Dr. John Reinhard, Dr. Raymond Dent, Dr. Kamal Henein, and Dr. Brandy Webb

John Reinhard, PhD, Head of R&D, with Diagnos-Techs™ staff analyzing bacteria using MALDI-TOF.

Digestive diseases affect 60-70 million Americans, account for over 100 ambulatory care visits and 13.5 million hospitalizations, and claim over 236,000 lives annually, according to statistics from the National Institutes of Health (http://www.digestive.niddk.nih.gov/statistics/statistics.aspx). Maintenance of intestinal health is greatly aided by the early, rapid, and accurate diagnosis of disorders. Towards that end, a variety of non-invasive tests have been developed that allow for testing on stool samples.

Among the causes of gastrointestinal disease, bacteria are key elements. We are normally host to a spectrum of bacteria that are of symbiotic importance. The collection of our resident bacteria, commonly referred to as the *microbiome*, can change when invaded by certain pathogenic strains of bacteria. Gastrointestinal infections affect more than 200 million people, resulting in 2.3 million ambulatory care visits, 450,000 hospitalizations, and over 4,300 deaths annually. Rapid and accurate detection of enteric pathogens can help diagnose and treat maladies before they progress. Classical microbiological testing often requires days for identification using the appropriate growth conditions. Recognizing the need for more accurate and faster detection, mass spectrometric methodologies have been adapted to aid in the identification of bacterial strains. A major breakthrough in such analysis came with the development of Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) mass spectrometry, which allows for the proteomic "fingerprinting" of bacteria. These analyses reveal a spectrum of proteins that have been assembled into databases against which candidate samples are compared. As a result, it is possible to accurately identify bacterial samples in as little as 11 minutes.

Continued on page 2.

Enhanced Gastrointestinal Laboratory Testing continued from front page.

Other technologies are also increasing the diagnostic power of the microbiology laboratory. The Vitek-2 system, for example, allows for both identification and antibacterial sensitivity assessments of candidate organisms.

Bacteria are a significant contributor to gastrointestinal pathology, including inflammatory conditions like inflammatory bowel disease (IBD). IBD is principally composed of two disorders, ulcerative colitis (UC) and Crohn's disease (CD). Both are autoimmune disorders in which cell-mediated immunity (principally T-cells) is directed against cells of the large and small intestine. Ulcerative colitis is a chronic inflammatory disease primarily affecting the colonic mucosa. The extent and severity of colonic involvement is variable. In the most limited form it may be restricted to the distal rectum, while in its most extended form the entire colon is involved.

The incidence of UC has been increasing in the western world since the early 1950's, largely attributed to better and more uniform diagnostic criteria. More recent trends indicate a change in the epidemiology of UC, with previously low-incidence areas now reporting a progressive rise in incidence. The western literature typically reports an incidence of approximately 6-8 cases per 100,000 and an estimated prevalence of approximately 70-150 cases per 100,000.

The definitive diagnosis of either Crohn's or ulcerative colitis is made by endoscopic examination. In addition to endoscopy, certain fecal tests can provide diagnostic benefit in the identification of these disorders. Since both Crohn's and ulcerative colitis are autoimmune disorders, inflammatory cells are found both at lesion sites and in the feces. Measurement of inflammatory cell markers provides a means of detecting active inflammation in the intestine. Examples of such markers include lysozyme and alpha anti-chymotrypsin.

Inflammatory Markers

Lysozyme

The two primary types of inflammatory bowel disease are Crohn's disease and ulcerative colitis. Collagenous colitis, lymphocytic colitis, and diversion colitis are more recently recognized inflammatory bowel diseases. Infection with parasites or bacteria may also cause gut inflammation.

Endoscopic evaluation with histopathological tissue sampling is generally considered indispensable in the investigation of a patient with suspected intestinal inflammation. Stool samples obtained during a sigmoidoscopy are needed to analyze for evidence of a bacterial or parasitic infection.

The human immune system defends the body against foreign substances using physical, chemical, and biological mechanisms. Such substances include microorganisms, such as bacteria, viruses, and fungi. The natural immune system is the first defense line comprising all mechanisms that resist infection. Despite a frequent exposure to a variety of potential pathogenic microorganisms, intestinal infection of bacterial agents rarely occurs in the gastrointestinal tract of a healthy subject. The membrane lining the digestive tract is among the physical barriers against invaders. This barrier is further defended by a non-specific antimicrobial system consisting of numerous peptides called *antimicrobial peptides*. Including lysozymes, these antimicrobial peptides attack the bacterial cell wall, causing loss of structural integrity and collapse of the bacterial cell. Lysozyme can be detected in many body tissues and secretions, including saliva, mucous secretions, and feces ultrafiltrate. In the gut, excess lysozyme may be found in diseased mucosa due to bowel inflammation. In active inflammatory bowel disease, elevated fecal loss of lysozyme is expected as part of increased enteric protein loss. It was found that lysozyme activity directly correlates with the clinical status and severity of the disease. Research shows that measuring fecal lysozyme concentration was helpful in differentiating normal individuals and patients with irritable bowel syndrome from patients with inflammatory bowel disease. Fecal lysozyme may therefore be used as a reliable tool both in diagnosis and in follow-up in the out-patient clinic for gastroenterology.

Diagnos-Techs™ offers a lysozyme test (MB3) as a stand-alone test or as part of a comprehensive gastrointestinal study (GI-01 and GI-02).

Alpha Anti-chymotrypsin

Alpha anti-chymotrypsin is a protein produced primarily during the acute phases of inflammation. It serves to prevent excess tissue damage that can result from proteolytic enzymes that are released by mast cells and neutrophils during the acute inflammatory response. Stool alpha anti-chymotrypsin levels increase at times of GI inflammation, particularly of the small intestine. High stool levels of this protein are typically associated with inflammatory bowel diseases, especially Crohn's disease (likely due to its tendency to affect the small intestine), but it can also be elevated in acute intestinal inflammation secondary to other causes.

Diagnos-Techs™ offers a test for alpha anti-chymotrypsin (MB4) as a stand-alone test or as part of a comprehensive gastrointestinal study (GI-01 and GI-02).

Microscopic Examination for Ova and Parasites

Microscopic screening of stool is among the oldest and most accurate methods for assessing the presence of both single-celled and multi-cellular parasitical infection within the GI tract.

The visualization of organisms via microscopy provides one of the most definitive forms of medical confirmation relating to the existence of infectious agents and is of particular value in relation to the diagnosis of gastrointestinal parasitic disease. Typically, samples are taken from multiple stool specimens collected on consecutive days. Mono-cellular parasites may be detected under light microscopy with the use of specialized staining techniques. The tiny and nearly transparent microbes become clearly visible when properly stained and provide the visual evidence for a trained medical microscopist to identify the organism in question, as well as its relative concentration. Multi-cellular parasites, including tape worms, round worms, and flukes, can be identified microscopically, and in some cases, by the naked eye. The gross presence of tape worm segments or entire bodies of round worms, for example, provides definitive evidence of infection. Microscopically, visualization of proglottids, eggs, or cysts, as well as adult fluke organisms, provides the specific evidence needed to identify these infectious parasites.

In some cases, relatively harmless parasites can appear so similar to their pathogenic relatives that other forms of testing, such as ELISA, are required to differentiate between them. An example of this would be in the case of Amoeba histolytica and Dientamoeba coli. The latter can exist without evidence of disease in the colons of some people. In many instances, it may be indistinguishable from Amoeba histolytica when viewed microscopically. In such cases, confirmational serologic or salivary antibody testing can resolve the question and support the diagnosis.

The ability to observe the microscopic contents of the GI tract also provides for quick identification of numerous pathogenic and potentially pathogenic organisms. Many times, combinations of these organisms lend clues as to the origins of infection, thereby facilitating the clinician's ability not only to treat the condition but to counsel the patient in ways calculated to avoid reinfection. The observer may also get a sense of the relative concentrations of the microbes in question. One organism that is the frequent cause of gastrointestinal dysfunction is Blastocystis hominis. While not unequivocally classified as pathological, its presence in moderate-to-large quantities evidences clear dysbiosis, usually associated with clinical signs and symptoms. Undigested yeast may appear in ova/parasite microscopic exams. These findings are typically associated with weak digestion and do no represent an invasive or infective state of the yeast organisms observed, the yeast being dietary in origin.

Diagnos-Techs™ offers stool analysis of ova and parasites (GP2) as a stand-alone test or as part of a comprehensive gastrointestinal study (GI-01 and GI-02).

Exocrine Marker

Chymotrypsin

Chymotrypsin is one of many secretions of the exocrine pancreas. Chymotrypsin is an enzyme that serves to break down proteins in the small intestine, thereby enabling their absorption. In cases of exocrine pancreatic insufficiency, chymotrypsin secretion declines, resulting in low levels of chymotrypsin in the stool. In pancreatic insufficiency, we see a decline in output of not only chymotrypsin but also other pancreatic secretions, such as amylase and lipase. This makes stool chymotrypsin a convenient marker of overall pancreatic output and thus, digestive capacity. Low stool chymotrypsin is frequently seen in acute or chronic pancreatitis, cystic fibrosis, diabetes (types 1 and 2), and functional exocrine pancreatic insufficiency where no organic cause is identified.

Diagnos-Techs™ offers a chymotrypsin test (FG1) as a stand-alone test or as part of a comprehensive gastrointestinal study GI-01 and GI-02).

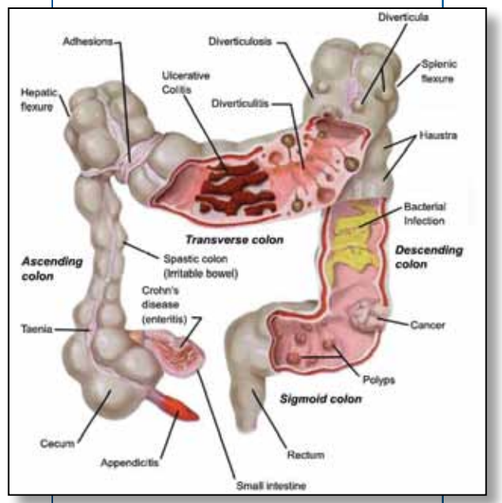
References on back cover.

Intestinal Microbiota and its Benefits

Dr. Kamal Henein

The human gastrointestinal tract is inhabited by a large and diverse bacterial community consisting of over 400-500 species, with the highest concentrations residing in the distal gut. Within the colon, population density approximates 1011 microorganisms per gram content, accounting for approximately 2% of a human's total body mass. Conversely, just 102 microorganisms per gram content exist in the proximal small intestine; these comparatively low bacterial concentrations derive from the effects of gastric hydrochloric acid, as well as biliary and pancreatic secretions. Relative to the intra-luminal environment of the colon, that of the small intestine is more dynamic; the effect of increased peristaltic activity progressing towards the colon results in a reduced transit time. Such conditions do not favor bacterial growth, as opposed to the relatively static state of the distal portion of the alimentary tract.

The initial establishment of the enteric flora starts soon after birth and is influenced by the method of delivery (vaginal versus cesarean section) and diet (breast versus formula feeding). In the case of breast feeding, the mother's own systemic health and nutritional status contributes significantly to both the initial inocculatory phase as well as propagation within the intestinal lumen. During the ensuing years, the enteric flora comes to outnumber human somatic cells by an estimated tenfold. Finally, the gut flora is also influenced by external factors, such as medications, geographical region, stress, lifestyle, and alcohol use. Although these factors clearly help direct how an intestinal bacterial community will evolve, host factors also play a role.



Recent medical research has evidenced the fact that the gut flora is individual-specific; that is, every individual has a unique combination of predominant species differing from that found in others.

The composition of the gut flora fluctuates under different circumstances, such as acute diarrhea, use of antibiotics, or dietary changes. Yet the pattern of gastrointestinal flora usually remains constant, providing further evidence that host factors are important. The intestinal flora plays an important role in the health of individuals; it has an influence in the morphological, immunological, and nutritional functions of the digestive tract. Consequently, it may be involved in many diseases, ranging from acute infectious processes to autoimmune disorders.

Fermentation of carbohydrates and non-digestible dietary residues constitute an important metabolic function of colonic microflora and a major source of energy for the host. Carbohydrate fermentation, with production of short chain fatty acids, results in a low glycemic response to oral glucose, a response consistent with increased sensitivity to insulin. The quality, quantity, and proportion of short chain fatty acids are modulated by enteric flora and diet, and can either improve or disrupt different functions of the host.

Enteric flora also play a part in absorption of calcium, magnesium, and iron, in addition to synthesis of vitamins (especially biotin inositol, folate, B12, and K). Another important role of gut flora on physiology of the colon is their trophic effect on the intestinal epithelium, inducing epithelial cell growth and differentiation in the large and small bowel.

Another interesting and important function of intestinal microflora is found in the interaction between host and bacteria at the mucosal level. Bacterial metabolites and antigens play a significant part in the development of a competent immune system. Local gut and even systemic immunity can be constantly modified by persistent host-bacterial interactions.

Intestinal flora forms a line of resistance to colonization by exogenous pathogenic microbes and enhances resistance to tissue invasion by pathogens (barrier effect). Non-pathogenic bacteria compete with pathogenic ones for attachment sites and nutrient availability.

Current medical research demonstrates that gut flora may play a determinative role in the development of certain pathological disorders, including colon cancer, inflammatory bowel diseases, immune mediated disorders, obesity, and irritable bowel syndrome. It is possible, then, through changing the makeup of the gut flora, to either prevent development of such disorders or to treat them when they occur.

Research has found some patients with inflammatory bowel disease to have lower diversity or depletion in beneficial bacteria. Supplementation with probiotics was shown to be helpful in these patients. In irritable bowel syndrome, data evidences reduction in bloating and pain after administering probiotics therapeutically. Observational studies in obese patients suggest that obesity is associated with substantial changes in the composition and metabolic function of the gut microbiota. Preclinical studies have demonstrated that changing the balance of bacteria in the gut through chronic probiotic consumption may alter a patient's emotional response to negative stimuli. Though this research is yet in its infancy, the implications of bacterial metabolites, especially the monoamine moieties, substantially affecting brain chemistry is intriguing and should constitute a subject of interest to the molecular biologist, neurophysiologist, and clinician alike.

Due to the increased interest in determining intestinal microbiotic composition for assessment of its metabolic, nutritional, and immune functions, Diagnos-Techs™ is introducing mass spectrometry identification for intestinal bacteria. This test is more than 97% accurate. It is included in the regular Gastrointestinal Health Panel (code GI-01) or the more comprehensive Expanded Gastrointestinal Health Panel (code GI-02), You can also order the stool culture for bacteria (code GP3) separately.

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Diagnos-Techs™ Lab News

Announcing Diagnos-Techs'™ Advanced Microbiological Testing and Research

Diagnos-Techs™, the leader in saliva-based hormone evaluation, is bringing advanced microbiological testing and research technology to its state-of-the-art laboratory facilities. Coming in Q1 2013, this $2M expansion includes MALDI-TOF mass spectrometry for fast, precise detection of over 4000 microorganisms, including E. coli, as well as the Vitek-2 system to measure bacterial sensitivity to antibiotics.

In addition to investing in next-generation technologies available in just five percent of U.S. medical centers, we've also staffed a new team of experienced microbiologists, making Diagnos-Techs™ the premiere resource for accurate, non-invasive testing and cutting-edge research.

For more information on our expansion and how it can help you improve patient health and well-being, please contact Diagnos-Techs™ at **1-800-878-3787** or visit our website **www.diagnostechs.com**.

Diagnos-Techs™ Introduces—

Director of Research and Development

John Reinhard, PhD

Dr. Reinhard studied chemistry with Dr. John Neumeyer at Northeastern University (Boston, MA) before attending the Massachusetts Institute of Technology, where he earned a PhD in biochemistry in 1980 under Dr. Michael Moskowitz. He was a post-doctoral fellow in Pharmacology at Yale University under the supervision of Dr. Robert Roth. Dr. Reinhard joined the Wellcome Research Laboratories in 1982 as a senior scientist and remained as a principal scientist through two mergers, ending as a clinical researcher at GlaxoSmithKline. In 2001, Dr. Reinhard co-founded Trelion Pharmaceuticals (now Avera) and later served as Senior Director of Clinical Research at Epix Pharmaceuticals (Lexington, MA), Associate Medical Director at Repligen Corp. (Waltham, MA), and Senior Director of Clinical Research at Ore Pharmaceuticals. In 2010, Dr. Reinhard co-founded Rhine Pharmaceuticals (Bellevue, WA) before assuming his current position as Director of Research and Development at Diagnos-Techs™. He has served on NIH advisory boards (intramural and extramural), has authored 76 peer-reviewed publications, and is listed as inventor on six patents.

Head of Microbiology Operations

Kelly Johnson

Kelly Johnson, initially from Grangeville, Idaho, attended Arizona State University and the University of Idaho, graduating in 1985 with a degree in Microbiology. Mr. Johnson began his career at the Laboratory of Pathology in the microbiology department of Seattle's Swedish hospital.

Mr. Johnson received his certification as a Registered Microbiologist in Medical and Public Health Microbiology from the American Academy of Microbiology in 1988 and certification as a Clinical Microbiologist from the American Society for Clinical Pathology in 1993. He received further training in Clinical Mycology from the Mayo Clinic in 1995.

In the aggregate, Mr. Johnson has more than 18 years of bench experience at Laboratory of Pathology/Dynacare, Polyclinic, and Northwest Hospital. He has served as a Supervisory Microbiologist for more than eight years.

In addition to his clinical experience, Mr. Johnson has taught Microbiology, Mycology, and Parasitology for more than eight years to students in the Medical Laboratory Technology Program at Shoreline Community College. He has also taught Basic Laboratory Science to Clinical Laboratory Assistant students at Edmonds Community College.

Consultant & Educator for Diagnos-Techs™

Nathan Goodyear, MD

Dr. Goodyear received his Bachelor of Arts from Louisiana Tech University in Ruston, LA, and Doctor of Medicine from LSU Health Sciences Center in Shreveport, LA. He is board certified in gynecology and is a Fellowship-Trained Metabolic Specialist (Anti-Aging/Regenerative Medicine). He was the chief resident in obstetrics/gynecology at the University of Tennessee in Knoxville.

Dr. Goodyear currently serves as a consultant, educator, and trainer for Diagnos-Techs™. Dr. Nathan Goodyear is the founder and lead physician at Seasons Wellness Clinic & Spa in Ruston, LA. His passion for wellness began with his own 100 pound, post-football career weight loss. He is dedicated to offering the latest advancements in traditional medicine with the most holistic approach to treatment possible.

Dr. Goodyear is also a partner and lead physician of Seasons Primary Care in Ruston, LA and Seasons of Farragut located in Knoxville, TN, and is a partner of Spring Media USA and Impact HealthCare Solutions, both located in Ruston, LA.

Upcoming Free Webinars:

January 10th, 2013 – *Avoiding Common Mistakes with Salivary Testing*

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Diagnos-Techs™, Inc. is a non-contracted provider with all insurance companies. Please verify your out-of-network benefits (including out-of-network deductibles and co-insurances) by contacting your insurance carrier. Diagnos-Techs™, Inc. will bill your insurance at the retail price per line item. If deductibles and/or co-insurances are applied, Diagnos-Techs™, Inc. is obligated by law in the State of Washington to collect.

HIPAA Compliance

The Health Insurance Portability and Accountability Act of 1996 (**HIPAA**) requires us to protect and maintain the privacy of our patients' identifiable health information. The standards are meant to improve the efficiency and effectiveness of the nation's health care system. We are committed to implementing appropriate administrative, technical and physical safeguards to protect the privacy of Protected Health Information. For more information, go to **www.diagnostechs.com** and click About Us/Notice of HIPAA Privacy Practices.

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- **Cotton Collection Tip:** To ensure adequate saturation of cotton rolls supplied in some of our test kits, instruct patients to wait until their mouths have refilled with the usual amount of saliva before removing and placing rolls in vials.

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Exciting Developments **continued from front page.**

to serology in diagnosing congenital toxoplasmosis in infants. It was Dr. Peyron's interest in availing non-invasive diagnostic tests to special patient populations that led him to study saliva-based immunological detection of pathogens. Among Dr. Peyron's projects is the advancement of diagnosis and treatment of tropical diseases in developing countries, an effort that has even led to support by the esteemed Gates Foundation. Dr. Peyron chose one other institution, the University of Washington medical school, to present his research findings before returning to France. As the laboratory that pioneered salivary diagnostics 25 years ago, we at Diagnos-Techs™ were honored to host Dr. Peyron, and we would like to extend our deepest thanks to Dr. Peyron for sharing his cutting-edge research findings with us.

Enhanced Gastrointestinal Laboratory Testing **continued from page 3.**

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