

DIARRHEA DIGEST

DIARRHEA DIGEST is an irregular publication of TECHLAB dedicated to the etiology, diagnosis, and therapy of diarrheal diseases and related aspects of intestinal ecology

Noninvasive diagnostic tests for assessing patients with IBD or IBS --- are the tests worth using?

There are over 25 million persons in the U.S. with irritable bowel syndrome (IBS), accounting for the majority of office visits to the gastroenterologist. It is estimated that only 10% of these people seek medical treatment. Even so, the 2.4 to 3.5 million visits to gastroenterologists for IBS not only overwhelm many smaller medical practices but put an excessive strain on the already burdened healthcare system. Although the incidence of inflammatory bowel disease (IBD) is much smaller, ranging from 1.5 to 2 million patients each year, these patients oscillate between active and inactive disease for life, needing continued medical therapy (1). IBD occurs not only in adults but can affect children younger than 5 years.

Diagnosing IBD typically involves multiple clinical assessments and a combination of expensive and invasive diagnostic procedures such as barium X-rays, colonoscopy, and biopsy for histological analysis (2). IBD, which is subdivided into Crohn's disease (CD) and ulcerative colitis (UC) may mimic other chronic intestinal illnesses such as diarrhea-predominant (IBS). The number of patients requiring a gastroenterologist and the associated medical costs are driving a new interest and need for rapid diagnostic tests for assessing

patients suspected of IBS and IBD.

Many IBD patients require a combination of diagnostic procedures for distinguishing between a flare or relapse of IBD and an infectious diarrhea or even episodes of IBS. Due to the combination of expense and invasiveness of current assessment procedures and the constant need for repeat patient evaluations, the use and need for rapid diagnostic procedures like immunoassays and lateral flow test are increasing in popularity. The more common rapid diagnostic tests are the serological assays for antibodies to *Saccharomyces cerevisiae* (ASCA) that are specific for Crohn's disease and for antibodies to neutrophil cytoplasmic antigens (ANCA) that are specific for ulcerative colitis. In a clinical study that included both adult and pediatric IBD with the testing being done at 5 different test centers, the sensitivity of the ASCA serum assays range from 39 to 44% and showed a specificity of 87%. The sensitivity and specificity for the ANCA serum assays range from 31% to 63% and 75% to 100%, respectively.

The poor sensitivity for IBD, along with the associated expense, discourage many medical centers from utilizing these assays as a first-step assessment tool. A more common approach is the use of ASCA/ANCA serum analysis for more difficult cases following more invasive procedures. The serum assays in combination do not

WARNING! This newsletter contains explicit intestinal information and portions may be rated BS for content. Readers are advised to proceed at their own discretion.

typically increase the sensitivity but significantly increase the specificity to 95% to 100% for both Crohn's disease and ulcerative colitis (2).

A more recent and novel approach for utilizing a panel of rapid diagnostic assays for assessing patients with chronic intestinal illnesses is fecal testing. Human lactoferrin is a glycoprotein that is present in most mucosal secretions and a primary component of the granules of activated neutrophils. During the onset of intestinal inflammation, activated neutrophils infiltrate the intestinal lumen causing an increase in fecal lactoferrin levels. The *IBD-CHEK* test is a qualitative ELISA that detects elevated fecal lactoferrin as an indicator of intestinal inflammation. The test results may be used as an aid to distinguish IBS from active IBD during the initial assessment of patients suffering from chronic intestinal illnesses. Clinical studies have shown that lactoferrin levels of healthy persons are similar to IBS patients while increased in patients with active IBD (3, 4). The test is both sensitive and specific for indicating active IBD.

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Comments or suggestions about the *DIARRHEA DIGEST* are greatly appreciated. If you would to be added to or deleted from the mailing list, please notify us.

Another rapid assay, the *IBD-SCAN* test, is a quantitative ELISA that measures the level of lactoferrin with results being reported as mg/mL of feces. These results are useful for monitoring disease activity in confirmed IBD patients. Changes in the level of fecal lactoferrin may be used to determine the clinical response to medical therapy and to predict a relapse. Recent clinical studies have shown that lactoferrin levels for IBD patients in confirmed remission return to baseline levels as seen in healthy persons. In addition, levels increase rapidly during active disease and even precede clinical symptoms by a mean period of 3 weeks (5). These studies confirmed our earlier results generated by monitoring the fecal lactoferrin of a co-worker's spouse suffering with ulcerative colitis. The *IBD-SCAN* test was used to follow the lactoferrin levels of this subject suffering from ulcerative colitis during a "flare" of active disease through remission (see Fig. 1). Lactoferrin levels reached almost 10,000 mg/g feces during the peak of active disease and dropped rapidly to 7 mg/g feces following anti-inflammatory drug therapy. The patient experienced a relapse showing a peak lactoferrin level of more than 1,500 mg/g feces. During remission (inactive disease), the patient showed a mean lactoferrin level of 11 mg/g feces (6; Fig. 1). The *IBD-SCAN* test results may prove useful for determining the effectiveness of medical therapies and for evaluating the utility of novel drug therapies.

Considering the speed and minimal cost for the noninvasive fecal testing, fecal lactoferrin tests offer gastroenterologists a new effective approach for the diagnosis of IBD and IBS. Even though these tests do not

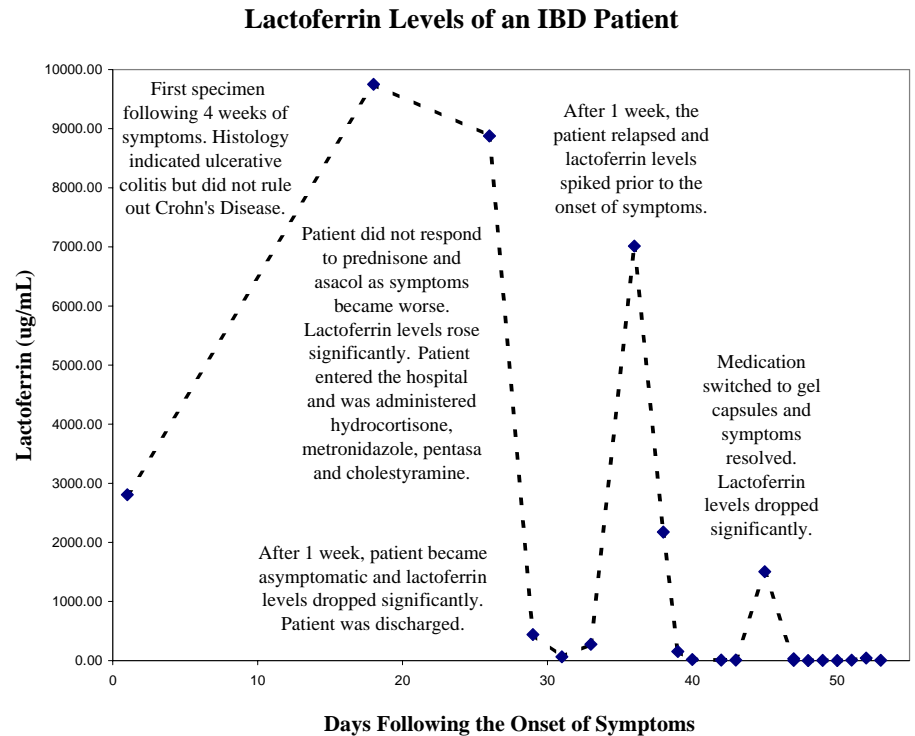
replace the traditional diagnostic methods, they can serve as useful tools used in combination as determined by the gastroenterologist. As additional rapid diagnostics tests are accepted as standard assessment tools, the question will not be if to use them but when.

James Boone

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6. Data on file at TechLab, Inc.

Fig. 1. The following graph shows how the level of lactoferrin closely followed a "flare" in an IBD patient and how the levels decreased following appropriate therapy.



TECHLAB Hot Sauces
Check 'em out on-line!

TORRID TUSH

HOT SAUCE

HINT: Keep toilet paper at 2-4°C for instant relief

5 FL. OZ. (148 ML)

Nutrition Facts	
Serving Size	Servings Per Container 30
Amount Per Serving	
Calories 0	
Total Fat 0g	0%
Sodium 120mg	5%
Total Carb. 0	0%
Sugars 0	
Protein 0g	
Vitamin A 6%	
*Percent Daily Values are based on a 2,000 calorie diet.	
INGREDIENTS: HABANERO PEPPERS, CARROTS, ONIONS, VINEGAR, GARLIC, LIME JUICE & SALT.	
SHAKE WELL	

HBO and IBS

The HBO series “The Sopranos” has broken new ground in many areas and this year they entered our area. Our new favorite character is Adrianna La Cerva. She is the fiancé of Christopher Moltisanti – Tony Soprano’s nephew. Christopher showed his love for Adrianna by purchasing the Lollipop Club for her – she immediately changed the name to Crazy Horse. To further complicate her life she is an informant for the FBI replacing Pussy Bonpensiero who got whacked. Small wonder that this highly stressed woman in her late twenties developed irritable bowel syndrome (IBS). This new development was introduced to the HBO audience first when she had to rush out of the car while she was talking to the FBI to get to a bathroom in a real hurry!

The writers had good medical advice and got the symptoms just right even down to Adrianna telling the doctor the consistency of the diarrhea ... very liquid. IBS is most common in young women under stress. The doc told her correctly it might be IBS caused by nerves or could be inflammatory bowel disease (IBD) with its more serious

consequences. Too bad we didn’t know the writers – maybe we could have gotten them to use TECHLAB’s *IBD-CHEKTM* to test her diarrhea for lactoferrin to rule out IBD.

Beetlejuice, Beetlejuice (Beetle Guano)

It’s old news that bat poop is mined by the hundreds of tons for fertilizer. What you may not know is that you are actually not buying bat dung at all but rather the dung of beetles that eat bat dung! That beetle poop is wonderful stuff for the garden or houseplants. A lot of it is harvested near San Antonio, Texas – a state known more for bull poop. The bats live in Bracken Cave and the dermestid beetles (*Dermestes carnivorus*) live below them, waiting patiently for the manna to drop from above. After the beetles and their microbes process the bat poop, it is harvested with a giant vacuum cleaner dropped through a hole in the roof of the cave while the bats are out shopping and then transported to a store near you. There are over 20 million bats in the cave – so how many beetles does it take to make over 170 tons of beetle poop every year?

Maybe diarrhea is not so bad!

We always thought that diarrhea was only good for people who study diarrhea. Let’s face it, if there’s no diarrhea, we may as well pack up and go home. At least that’s what we used to think, until we read a piece of news in The Scientist: “Colon Cancer Resistance Found in Traveler’s Diarrhea.” The news article referred to a report in PNAS by Pitari et al: Bacterial enterotoxins are associated with resistance to colon cancer (Vol 100:2695-2699). The authors demonstrated that a bacterial heat-stable enterotoxin was able to suppress colon cancer cells from proliferation. The mechanism of this suppression is rather complicated. It covers several aspects including elevation of intracellular cGMP by the enterotoxin, induction of an L-cis-diltiazem-sensitive conductance, and subsequent promotion of calcium influx and inhibition of DNA synthesis in the cancer cells. *The bottom line?* Diarrhea may not be that bad for a patient suffering from it --- that’s easy for us to say! *The challenge?* Find out which patients with diarrhea may be getting their colon cancer treated.

“Everyone has to Poop ... Even your Mummy”

The ability of TechLab’s *E. histolytica II* kit to detect the protozoan parasite *Entamoeba histolytica* in human feces has recently undergone its most rigorous evaluation to date. In a study published in Transactions of the Royal Society of Tropical Medicine and Hygiene¹, the kit was used to identify *E. histolytica* in desiccated feces recovered from mummies and cesspit sediment dating back 5300 years. The authors utilized the ELISA based assay to detect preserved antigenic epitopes specific to the cell-surface adhesion lectin of *E. histolytica*.

The study of parasites, their prevalence, and their distribution in archaeological material is termed paleoparasitology. Work in this field has described human infection with *Giardia sp.*, *Plasmodium sp.* (the causative parasite of malaria), and *Trypanosoma cruzi* (the causative parasite of Chagas disease) in ancient populations. Similar work by paleomicrobiologists has examined ancient outbreaks of microbe-related diseases such as mumps, tuberculosis, and the plague. The current study by Goncalves and co-workers is the first to describe the widespread distribution of *E. histolytica* infection throughout antiquity. The authors evaluated desiccated stool samples, termed coprolites, isolated from naturally mummified human remains and archeological excavations of human latrines. Identification of protozoan parasites from such material is complicated by the fact that organisms in all stages of the life cycle, including cysts, decay rapidly under normal environmental conditions. The authors theorized that, although the integrity of whole-organism morphology may suffer, specific antigenic epitopes may be preserved that would allow for identification of infecting parasites.

The study demonstrated *E. histolytica*-positive feces in 100-5300 year old samples taken from Argentina, Belgium, France, Switzerland, and the United States. Since TechLab’s *E. histolytica II* kit utilizes a monoclonal antibody specific for the pathogenic parasite *E. histolytica*, and does not cross-react with non-pathogenic *Entamoeba* species such as the morphologically similar *E. dispar*, it is likely that individuals in these populations suffered clinical manifestations of *Entamoeba* infection. However, the authors did not comment on related morphological findings, or potential causes of death, for the mummified humans that tested positive for *E. histolytica* infection. Most specifically, the study demonstrates that analysis of fecal remains for pathogen-specific antigens can be a useful tool for describing historical disease trends.

While interesting, the study does raise the question of how paleoparasitological and paleomicrobiological screenings relate to modern intestinal ailments and modern medical practice. Since conventional wisdom imparts that, “those who ignore history are doomed to repeat it”, studies such as this must be useful beyond simply characterizing disease distribution throughout history. Importantly, these studies can help to map the genetic evolution of disease, outline societal trends that support disease, and describe the ability of different medicinal strategies to control or eradicate diseases.

These theories concerning the usefulness of paleoparasitology relate directly to the current study by Goncalves and co-workers. The antibodies specific to *E. histolytica* used to evaluate the samples have identified the pathogenic variant of *Entamoeba* as a likely human parasite for over 5300 years. This indicates that *E. histolytica*’s evolutionary divergence from *E. dispar* is not a new occurrence.

However, more specific techniques, such as PCR, should be employed to answer this question definitively. The study also suggests that cesspools, or drinking water contamination by cesspools, may have contributed to the spread of the disease in ancient civilizations. Finally, comparison of disease rates in ancient populations to their medicinal practices may lead to future treatments and cures for *E. histolytica* infection. Such in-depth analysis of the current study, and similar studies, related to *E. histolytica* infection is relevant to the medical community today. The World Health Organization estimates that there are 50 million cases of *E. histolytica* infection and 100,000 deaths caused by infection each year, with the vast majority occurring in developing countries². Many of the causes for disease progression and transmission in those countries are similar to the ones found in ancient societies. Clearly, improved detection methods, treatments, and

preventative measures are needed in order to reduce the detrimental impact of *E. histolytica* on human health.

TechLab is continually working on new products to improve the detection of *E. histolytica* infection. Perhaps, the current study examining desiccated feces will entice us to re-think our recommendation for using “fresh” stool samples for tests run with our *E. histolytica II* kit. If we begin validation procedures immediately, we may be able to corner the market on analysis of 5300 year old mummified fecal remains. Expect the results to be out in May or June of 7304.

Joel Herbein

¹ Goncalves ML, da Silva VL, de Andrade CM, Reinhard K, da Rocha GC, Le Bailly M, Bouchet F, Ferreira LF, Araujo A. 2004. Amoebiasis distribution in the past: first steps using an immunoassay technique. *Trans. R. Soc. Trop. Med. Hyg.*, 98(2):88-91.

²Tanyuksel, M, Petri WA, Jr. 2003. Laboratory diagnosis of amebiasis. *Clin. Microbiol. Rev.* 16(4):713-729.

Hot Dung Cause of Global Warning

A very big dark cloud floats just downwind of India intercepting the sun on its way to the ocean. Its been dubbed the “Asian Brown Cloud”. A United Nations agency warned that the cloud could “lead to several hundreds of thousands of premature deaths”. Volcanic ash? Industrial pollution? Buzzards? Nope – it’s smoke from cow dung fires. Seems that with all the sacred cows roaming around India there is a lot of dry cow dung and it makes a reasonable cooking fire. With hundreds of millions of dung cooking fires pumping soot and pollutants into the sky it can get more than a little pungent. From our reading --- the effects of the cloud on weather downwind is more than a bit controversial and is a political issue so we won’t go there -- - it’s just when rainwater tastes like ___ ; readers of *Diarrhea Digest* will know the reason and when the oceans rise to cover Florida, you will know why.

Our T-shirts may be tasteless but ...

Customer: “I was checking out TechLab, Inc., in connection with getting ready to do some business with them, and found this page about T-shirts on their website. Pretty funny. Too bad they are all sold out.”

Response from a second customer: “That has got to be some of the crudest, tasteless, most disgusting examples of bottom-of-the-barrel humor I have ever seen. Whoever came up with that stuff is a total sicko. It makes me want to throw up at the mere thought of what some people will sink to just for a cheap laugh! So, how long before they are re-stocked?”

Shut Up and Drink your Worms ...

Several issues ago, *Diarrhea Digest* carried an article by Dr. Joel Weinstock at the University of Iowa on his work with worms and their possible utility for treating inflammatory bowel disease (IBD). Dr. Weinstock and colleagues are continuing their intriguing research on this phenomenon and how worms are able to reduce the inflammation that occurs during IBD. There also is collaborative research by Dr. David Elliott and colleagues at the University of Iowa showing that helminthes protect mice against experimental IBD. Helminthic worms are common in warmer climates and have shared our intestines with us during our evolution. As a result, these researchers believe that our intestinal immune response has adapted to the presence of these worms, which have an innate ability to help control the body's response that leads to too much inflammation. So far, experimental treatment in humans has shown that a single dose of parasite eggs resulted in improvement in IBD patients, and no side effects have been noted.

Clinical studies are now being organized, and the work is being done under a strictly controlled research protocol. Several different worms, including the

porcine whipworm *Trichuris suis* and *Schistosoma mansoni*, are being tested. Porcine whipworms are at the top of the list because they don't survive very long in humans. The work has been taken to a new level now with the development of a drink that contains thousands of whipworm eggs, based on recent results showing that the drink reduces abdominal pain, bleeding and diarrhea associated with IBD. Soon the drink, called TSO (short for *Trichuris suis ova*), may be on sale in Europe if approved by regulators. The drink will be made by BioCure, a German company, and an application has been filed to the European Agency for the Evaluation of Medicinal Products for an EU-wide license. The drink would be taken twice a month.

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Think you've had a bad day at work???

A fellow who works underwater had to do a job when the water was very cool. The company who employed the diver used a diesel powered industrial water heater. The heater is a \$20,000 piece of equipment that sucks the water out of the sea, heats it to a nice warm temperature, and then pumps it down to the diver through a garden hose taped to the air hose. The diver would typically take the hose and stuff it down the back of his neck, flooding the whole suit with warm water, making it feel like a Jacuzzi. In this particular instance, the work was going well until all of a sudden, the diver's butt began to itch. So he scratched it. This only made things worse and within a few seconds, his whole rear end was burning and on fire.

The diver pulled the hose out of his back, but it was too late. What had happened was the heater had sucked up a jellyfish and pumped it into the diver's suit. With the hose stuck down the diver's back, the jellyfish had gotten caught in the diver's crack. When he scratched, he basically was grinding the jellyfish into his anus. The diver communicated his predicament to the dive supervisor, who had trouble understanding the diver's dilemma because he and the other divers were laughing hysterically. The dive was immediately aborted, but it was 35 minutes before the diver could get to the surface for his chamber dry compression. When he got to the surface, the diver was wearing nothing but his brass helmet. The suit and gear were tied to the bell. The on-board medic, who was laughing so hard that he was crying, handed the poor diver a tube of cream and told him to shove it "up his a—" when he got into the chamber. The cream put out the fire but the poor diver couldn't defecate for two days because his exit tube was swollen shut.

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