TECHLAB®

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

Fall 2008

Clostridium difficile diagnostics – Who moved the cheese?

When we started our research on this disease in the late 1970s, the research question was simple: What is causing the pseudomembranous colitis that is killing people who are taking clindamycin? The research teams collaborating on solving that maze ended up with a single organism, *Clostridium difficile*. The maze was solved by using a tissue culture assay to follow the "scent" to the "cheese". Feces from patients who developed pseudomembranous colitis caused human cells in tissue culture to retract and become rounded. This "rounding" of tissue culture cells was inhibited by antiserum made to the toxins of *Clostridium sordellii*. The "scent trail" first led to *C. sordellii*, but that organism could not be isolated from the feces. Later we learned that *C. difficile* shares similar toxin genes and that the antisera cross-reacts. The tissue culture assay could be done either with *C. sordellii* antiserum or antiserum prepared to *C. difficile* toxins. Either way it was 99% specific and sensitive for detecting pseudomembranous colitis in hospitalized elderly patients previously treated with antibiotics. The tissue culture assay remained the "gold standard" for diagnosis even as immunological assays for the toxins arrived on the scene. The tissue culture assay is exquisitely sensitive to toxin B and one picogram will cause cells to round up and start to detach from the plastic in 48 hours. During the 1990s, the most sensitive one-hour ELISAs detected about 92% of the patient samples that would become positive two days later in the tissue culture assay. Most clinical laboratories switched to the faster ELISA.

When the cause of the disease was discovered, the name was changed from the descriptive "pseudomembranous colitis" to *C. difficile* colitis or "*C. difficile* associated disease". The original name of "clindamycin colitis" was no longer used (much to the delight of Upjohn, the pharmaceutical company that made clindamycin). *C. difficile* colitis was described as an inflammation of the colon mucosa resulting in the formation of eruptive "pseudomembranes" formed by the deposition of fibrin and white blood cells at the individual sites of the inflammatory reaction to the toxins. The toxins appeared to initiate the damage to the colonic mucosa but the progression to pseudomembranous colitis was mainly an inflammatory response of the patient to the toxins and to the damage caused by the toxins. The disease occurred in hospitalized elderly patients who had received antibiotic therapy and had subsequently developed antibiotic-associated diarrhea that then progressed to colitis. The antibiotic-associated diarrhea was not caused by *C. difficile*, but the elimination of the normal flora allowed the *C. difficile* spores from the contaminated hospital environment to grow and produce toxin. In the normal colon the bacterial ecosystem has evolved to use every scrap of energy and there simply is no "food" left over for *C. difficile.*

Antibiotic-associated diarrhea is a very common occurrence and usually is a nuisance rather than a life-threatening problem. The general cause is the reduction of bacteria in the gut - but how the elimination of the bacterial ecosystem results in diarrhea is a matter of some debate. One theory is that when bacteria are not available to deconjugate bile salts in the terminal ileum, these detergent molecules reach the colon and cause diarrhea. The osmotic theory is that the lack of degradation of both small and large molecules in the colon results in an osmotic gradient that does not allow water to be pumped out of the colon. And of course there is always the possibility that an unknown pathogen is the cause. Whatever the reason, it is important to understand that *C. difficile* is not the cause of diarrhea in the majority of hospitalized patients and certainly

not in out-patients. Currently, about 15% to 20% of stools sent to clinical laboratories are positive by tissue culture or immunological tests for the toxins of *C. difficile.*

Until the last few years, *C. difficile* colitis was a result of elimination of the normal flora with antibiotics, ingestion of *C. difficile* spores from the contaminated environment, and growth of the organism when the level of antibiotics declined below the inhibitory level for *C. difficile*. *C. difficile* often could not successfully grow and produce toxin until several days after antibiotics were stopped and the level of antibiotic in the colon dropped significantly. Recently that has changed with the development of the new "outbreak" strains. Most of the publicity has been about a new super strain that produces 20 times as much toxin and kills patients faster than the "old" strains. We have not found that this strain (really a cluster of very similar isolates) produces more toxin than many of the "old" strains, but it does produce more than average. This group of isolates has a deletion in the regulatory gene that controls synthesis of the toxins so they may produce more toxin early in the growth cycle in the colon - but we have not been able to convince ourselves of this from our test tube experiments. Unlike "hyper-toxic" strains isolated before 2000, this strain is very resistant to fluoroquinolones. Indeed, many strains of *C. difficile* have become resistant to fluoroquinolones and can grow during therapy. This gives them a tremendous advantage and, in our opinion, is the major reason for the changing dynamics of the disease.

***DIARRHEA DIGEST* is published by TECHLAB®, Inc., 2001 Kraft Drive, Blacksburg, VA 24060-6358**

TEL: In the U.S., 1-800-TechLab
Outside the U.S., 540-953-1664
FAX: 540-953-1665
Email: techlab@techlab.com
Website: www.techlab.com

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

"*C. difficile*-associated disease" has changed. *C. difficile* used to be considered primarily a disease of elderly, hospitalized patients. This still holds true, but the patient population is no longer inclusive only for these patients. There are a few reported cases of otherwise healthy young adults coming down with colitis from *C. difficile.* Family-practice physicians are now aware of the disease and some seemingly send every soft stool for analysis. Ten years ago, a young "healthy" adult with mild diarrhea would not have been tested for *C. difficile*. We examined the demographic data collected over a 4-year-period from over 13,000 specimens submitted to a healthcare system that utilized a central lab for regional hospitals and family practices. This large healthcare system covers >800,000 patients. We found that about 15% of the stool samples were from patients younger than 40 years of age. Surprisingly (at least to us), only about half the samples were from hospitalized patients. Roughly 30% of the specimens were from outpatients and 20% were from nursing home patients. Only 30% of the specimens took the form of the container and 15% were hard stools. A few years ago well over 50% of the samples submitted for *C. difficile* testing would have been liquid. From another diagnostic lab we found that the mix was different with 70% of the stool samples coming from hospitalized patients, and 60% of these specimens were liquid.

As the sample mix has changed, the correlation of immunological tests to tissue culture results has declined in some, but not all, laboratories. Tests that were originally over 90% in agreement are now 70-85% sensitive in some situations and tests from some manufacturers are much worse. But the sensitivity of the tests varies with the patient mix. The reason is simply that more stool samples are submitted that are not "liquid" and that have very low amounts of toxin. These tests give 85 to 90 percent sensitivity for hospitalized patients with diarrhea. They are less sensitive if the sample is from a young out-patient with semi-solid stools. In general, the amount of toxin in formed stools from patients is usually very low. Patients on therapy for *C. difficile* are

sometimes tested everyday and sometimes multiple times per day. Obviously, some of these samples will contain very low amounts of toxin. Samples that are only positive after 48 hours of incubation in the tissue culture test are the ones most often missed by the immunological tests. Do we need to detect such low amounts of toxin? Does it correlate with disease? Should formed stools be routinely tested? Are the immunological tests positive for samples taken from these patients two days later when the tissue culture results are finally ready? These are questions that we hope clinical researchers will focus on solving.

The name of the disease seems to be changing once again. We increasingly see it referred to as "*C. difficile* disease". The subtle inference is that if you have "*C. diff*" you have the disease. That certainly was not correct in the past and it is not correct today. When I (along with many other investigators) first isolated this organism from the feces of patients with pseudomembranous colitis, I looked up what was known about the organism. *C. difficile* was described as a component of the normal flora of babies that produced a toxin that did not seem to cause harm; so I ignored it! I haven't examined maternity wards for several years but it was not uncommon to find that 50% of the babies in some hospitals had *C. difficile* and positive tissue culture results. They were doing quite nicely! Why weren't they affected by the toxins? No one knows for certain. Young people in general are less susceptible to the toxins and compromised older patients are the most likely to be affected. There are very few simple things about this disease.

There is a real desire to simplify this complex disease. "*C. diff* disease" implies that the mere presence of a toxigenic strain of the organism is sufficient cause to treat the patient with metronidazole or vancomycin. Charts are annotated with "test for *C. diff*, if positive give metronidazole." This has driven the desire to push diagnostics into the PCR arena where the presence of the genes for the toxins or the regulator gene will be sufficient to diagnose the patient as having "*C. diff* disease". Recently Prodesse announced their real-time PCR test for toxin B, ProGASTRO ™ Cd. In their 2008 product insert they claim detection of the toxin gene when 10^4 cells per mL (only 50 cells per reaction) of toxigenic *C. difficile* are present. They show an 86% correlation with tissue culture and 93% correlation to the REMEL toxin ELISA. This data certainly does not present a very convincing argument for going to the trouble and expense of doing PCR on feces. But like immunological assays, PCR tests from different manufacturers vary in sensitivity. PCR is a very sensitive assay – in some cases it could be too sensitive. A recent paper from the Mayo Clinic on a real time PCR method showed that it was "positive" for twice as many fecal samples as several commercial immunological test kits. This was done with ELISA and rapid assay formats from several manufacturers. The results, unfortunately, were not compared to the accepted gold standard – tissue culture. Even if we assume that the immunological tests were only 75% sensitive as compared to tissue culture, this PCR test will yield a 50% increase in positive samples that would be negative when tested by tissue culture. Presumably, this test must detect even fewer cells per gram than the Prodesse test. At its most sensitive, a PCR test with enough cycles could, in theory, detect a single spore. No one would argue that this would be useful – but where would you draw the line? Are the patients that are now being "missed" by tissue culture and immunological tests just carriers? Are they patients with minor disease that are getting well on their own? Do most of them progress to "detectable" disease; are they at risk?

So the cheese keeps moving around in the maze. What is the correct solution? The answer is easy – no one knows. Nothing can or should replace the clinicians' thoughtful diagnosis; they have to judge the patient's condition and then use laboratory results as an aid. Lab results are seldom the end all in any diagnosis and the same should be true for *C. difficile*. We know that even if the toxin is present in high amounts that some patients and most babies are not affected. It is our opinion that too many people are currently being treated for "*C. diff* disease" because clinicians don't make a thoughtful decision

but rather reflex to the "treat if present" mentality. *C. difficile* colitis can be caused by metronidazole or vancomycin just as it can be caused by almost any antibiotic. So clinicians could be causing disease by treating patients who don't need treatment. About 20% of patients who have symptoms and are treated with these drugs relapse; how much disease will be caused from treatments given to patients who don't need treatment? If we greatly increase the numbers of patients who are treated, will this help or hurt? Again no one really knows.

This field of work is in great need of carefully controlled clinical studies to answer these questions and more. Are the patients that have very low levels of toxin (below detection by tissue culture assays) in need of treatment or should they be watched carefully and treated only if the symptoms indicate colitis? How about the ones that are now missed by many of the immunological tests? How many would progress to colitis? How would you want your mother to be treated?

T. D. Wilkins

Hey you guys undergoing a colonoscopy --- take advantage of the situation and have your gastroenterologist write a note to your wife saying that *"Your head is not up here. We looked!"*

Got … fermented milk?

Fermented foods may mean "spoiled" to a non-microbiologist, but fermentation gives us some pretty good "by-products" --- cheese, cider, kimchi, olives and pickles, sauerkraut, soy sauce, and vinegar --- oh yeah, beer and wine --- to name a few. Yogurt, a soured milk product, is one of the most popular fermented foods. It's been in and out of the news for many years because of its probiotic benefits --- it helps us maintain a healthy intestine.

Yogurt, by definition, includes *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus.* For "health benefits", yogurt is often supplemented with *Lactobacillus* sp. The health benefits have been "dogma" for many years, but they were really brought to the public's attention more than a hundred years ago by the Russian biologist Mechnikov, who observed that Bulgarian peasants who ate lots of yogurt lived to very old ages. Mechnikov concluded that the *Lactobacillus* spp. were the reason. Whether yogurt increases our lifespan is debatable, but there is little doubt to its potential health benefits. Test results show that probiotic organisms in yogurt make beneficial enzymes, help us adsorb our vitamins and minerals, provide us with a good source of nutrition, regulate the level of acidity in our digestive tract, and provide anti-oxidative activity to get rid of radicals. This isn't just a bunch of hype thrown out by manufacturers; this has been known for a long time. In today's health-conscious world, micro-biologists are becoming even more vocal on the benefits of using good bacteria to fight bad bacteria.

It is likely that yogurt's "probiotic" effect will extend beyond the walls of the intestine. Down the road we may find that probiotics reduce our allergies. Recent studies showed that mice which have their intestinal flora modified by antibiotic treatment and then colonized by *Candida* developed allergic airway disease in the lungs. However, animals with healthy intestines did not. The ability of probiotics to keep our intestines healthy, coupled with the fact that our immune system is very much in touch with our intestinal flora, suggests that probiotic yogurt may be able to curb our allergies.

Yogurt has been in our diet for thousands of years. More than 4,500 years ago, our ancestors liked the sour tangy taste and lumpy consistency of milk stored in goatskin bags and put it into the recipe file. Fermented milk became a staple in many cultures.

The popularity of yogurt took off in the 1900s when its sour milk taste was made more palatable by a young man named Carasso. In the early 1900s, Carasso industrialized the process of making yogurt. He formed a company in Barcelona and named it Danone ("little Daniel") after his son. During World War II, Carasso moved his company to the U.S., began to incorporate fruit in his product, and Americanized the name Danone to Dannon. The rest is history.

Now we have all kinds of sweetened yogurt. Name a flavor, and you've got a yogurt for it. If you don't want yogurt, there are chocolate bars that contain more live active cultures than yogurt, less sugar, a good supply of calcium, and only 100 calories to boot. If you like cereal, you can get whole grain with live active cultures, a good dose of iron, no salt or trans fats, and a variety of flavors. For parents concerned about intestinal health for their toddlers, probiotic drops are available that can be given with a spoon or added to food and drinks. If you're on the go and can't take the time to stop and eat a container of yogurt, simply chew a tablet containing 100 million *Lactobacillus reuteri* cells.

Yogurt and similar concoctions are made in practically all cultures around the world. In Europe, plain yogurt is highly popular while in the U.S., flavored yogurts are the rage. In central Asia, a drink called kefir is made using fermented milk from sheep, goats, or cows. If horse milk is used, the product is called kumis. The milk is mixed with kefir grain, which is a combination of bacteria and yeast mixed with proteins, lipids, and sugars. The grain itself, once it develops, looks like a small head of cauliflower, although it can be as small as a grain of rice. The grains represent a microcosm of microorganisms. Fermentation proceeds at ambient temperature usually overnight. The product is sour, carbonated with some alcohol, and it has a thin yogurt consistency. It can be made in beer bottles to keep it carbonated. Kefir provides folic acid and can aid in lactose digestion. Other variations can be made depending on the composition. Water kefir, for example, is "grown" with water, sugar, dry fruit, and lemon juice.

Kumis, also popular in central Asia, is similar to kefir but is produced from a liquid starter culture using horse's milk. Kumis has higher alcohol content than kefir because horse's milk contains more sugars available for fermentation. For industrial scale, and because horse's milk is a limited commodity, cow milk supplemented with sucrose is often used. According to some legends, you don't want to drink unfermented horse's milk since it supposedly is a strong laxative. However, in that part of the world, horse's milk may be used as a substitute for persons who have allergies to cow's milk. Supposedly, George Bush tasted kumis when he visited Mongolia in 2005, but we never heard if he liked the drink. The kumis drink is served cold and is sipped. Traditionally, if you don't drink all of your kumis, it is poured back into the storage container so that future visitors will have enough to drink.

The key for a good probiotic is to get hundreds of millions of the good bacteria through the acidic environment of the stomach, through the small intestine to their final destination --- the large intestine. This isn't easy because we have evolved stomach acid as a protective mechanism to kill bad bacteria. However, companies are working on new pill coatings to protect the bacteria as they travel through the intestine.

Homeostatic soil organisms (HSO for short) are a recent addition to the growing number of probiotic bacteria. These organisms are isolated from unpolluted soil and plants, and are being checked for their potential health benefits as probiotics. Why use these soil bacteria? Because they are more stable than the ones currently used and can survive sitting on the shelf at room temperature for years. In addition, it looks like they are hardy bugs and can survive the stomach environment better than other more common probiotic organisms. HSOs will be used in the same manner as *Lactobacillus*, *Bifidobacterium*, and other probiotic bacteria --- to treat diarrhea, gas and bloating, and in general, compete with the pathogens.

Although the field of probiotics has a long history, the science has lagged. Efforts are underway to answer some nagging questions. For example, it still is unclear whether the probiotic bacteria in yogurt grow in the intestine, or whether they merely persist long enough to provide a service. Also, some yogurt bacteria probably bind to intestinal mucus glycoproteins, but whether they bind to intestinal tissue remains unclear. If yogurt bacteria can penetrate the mucus, then binding to the intestinal mucosa is possible. If so, this may be critical in how well the organism provides health benefits.

Some probiotic organisms are better at certain functions than others. Some may

modulate intestinal inflammation, some may be more antagonistic against enteric pathogens, and still others may affect intestinal permeability. There probably will be a time in the not-too-distant future when probiotics are tailor-made for particular illnesses and conditions. For example, *Lactobacillus* GG appears to reduce Crohn's disease in infants but not in adults. Some nonpathogenic *Escherichia coli* help maintain remission in patients with ulcerative colitis. To boost your immune system, *L. reuteri* and *L. rhamnosus* GG may be the key. *L. rhamnosus* GG also reportedly helps with eczema and allergies, and with irritable bowel syndrome (IBS). *Bifidobacterium infantis* may help with IBS.

In October, 2006, the "First Probiotics Symposium on Developing Probiotics as Foods and Drugs" was held at the University of Maryland. There was input regarding the status in the U.S. on the use of probiotics as foods and drugs, indicating that manufacturer's claims for health benefits likely will undergo more extensive review. This also may mean that concerns about the particular strains being promoted will undergo additional scrutiny. Some strains of lactobacilli, for example, are naturally resistant to vancomycin. The transfer of this trait to potential pathogens (e.g., *Clostridium difficile*) could lead to therapeutic consequences. To avoid this problem, leading scientists have suggested that probiotic organisms be susceptible to a minimum of two major antibiotics. In addition, scientists are requesting that strains be well-characterized at the molecular level for speciation and genomic DNA fragment analysis for accurate identification.

In today's World, we take massive amounts of broad spectrum antibiotics. We eat meat from animals treated with antibiotics. We drink water that has been chlorinated to kill bad bacteria. All of these efforts have improved our health and hygiene, but they kill our flora that has evolved with us over millions of years to protect us. The best way to respond is the way being touted by microbiologists. Use good bacteria to keep the bad ones away!

Stool Notes

You may have been on the hot seat before, but not like this! From CNNMoney.com, a Japanese manufacturer of high-tech toilets, complete with heated seats, air purifiers, blow dryers, and water sprayers, had three of the units catch fire.

Keep your bathroom interesting and fun! Toilet tank aquariums (Fish 'n Flush, located at www.fishnflush.com) and toilet tattoos ("The only way to crown your throne", www.toilet-tattoos.com/) will bring a crowd to any bathroom --- if that's your type of party.

Featured in the 2007 Darwin Awards - the story of an alcoholic who liked to take his liquor rectally. The fellow was addicted to enemas and on one occasion (his last), he took in 3 liters of sherry. He passed out from the enema, but his colon continued to absorb the sherry, resulting in a blood alcohol level of 0.47%. The poor guy died from alcohol poisoning and basically embalmed himself.

Toilet-to-tap (www.slate.com) - Instead of "don't drink the water", it's more like "don't think about the water". This is a "green" idea that recycles wastewater and sewage through expensive piping and aquifers. The concept is especially promising in dry areas because it brings cleaned-up water back in the kitchen tap and through the showerheads. In southern California, this is the world's largest water purification project to "turn on" water faucets while trying not to "turn off" folks with thoughts of drinking "poop" water.

Wedding dresses made of toilet paper were featured at Times Square in June --- the big month for weddings. Descriptions in the "Wedding" section describing the bride's dress might be interesting. The bride wore a smooth, two-ply, ultra-soft, absorbent wedding dress … (CNN.com/US)

Innovative Diarrhea Testing ---
Why worry? Be sure with the new DIARRHEA-CHEK! 100% accurate

TECHLAB --- #1 in the #2 business!

RAPID DIARRHEA-CHEK

A Point-of-Care Dot Diagnostic

****Collect "Midstream Sample" from appropriate orifice into box (Discuss any questions about your appropriate orifice with your physician)***
Be certain to grasp the box firmly with both hands (one size fits all)

If pee in box, then dry box with hair dryer and repeat

If miss box, get assistance and repeat (see Common Problems)

After collecting the specimen, replace the lid (quickly)

Shake vigorously (vortexing is not necessary and it might be dangerous)

Rattle → No Diarrhea (you may consider adding some fiber to your diet)

No Rattle → Rotate at 30° to horizontal 2 complete rotations → Remove Lid (gently)

< 3 dots Brown	3 dots Brown	4 dots Brown
No Diarrhea (or not enough to worry about)	***Indeterminate (Buy another kit)***	***Positive for Diarrhea --- watch out***

Another Innovative Quality Product from

TECHLAB

2001 Kraft Drive
Corporate Research Center
Blacksburg, VA 24060-6358

***If you did not make it to the bathroom, don't bother with the test – you have diarrhea**

T. D. Wilkins, Patent Pending

TECHLAB®

Rapid Intestinal Diagnostics and Intestinal Microbiology

TECHLAB, INC.
Blacksburg, VA

TECHLAB®

Rapid Intestinal Diagnostics and Intestinal Microbiology

www.techlab.com

2001 Kraft Drive
Blacksburg, VA 24060-6358

Your Copy of DIARRHEA DIGEST

TECHLAB's Technical Support Team is available from 8:30 AM to 4:30 PM EST, Monday through Friday
Call 1-800-TECHLAB (Outside the U.S., call 540-953-1664) or FAX 540-953-1665