Clostridium Species Reported in Stool Analysis at DDI

by Chuck Masur, MD and David Quig, PhD

Many DDI clients have asked for more information on the various clostridium species that may occupy large portions of the ecosystem that is the human gastrointestinal tract. In response, DDI will assist the clinician with a stepped approach to addressing this issue. Non-pathogenic clostridium species now are reported in the “beneficial/expected” section of our standard microbiology panel. We will continue to offer our Clostridium difficile Toxins A and B panel as an accurate assessment for the presence of C. difficile disease. In addition, DDI is pleased to announce the roll-out of the Comprehensive Clostridium Culture panel that includes identification of more than twenty expected and dysbiotic clostridium species. These cultures are performed under anaerobic conditions using special media optimally suited for the growth of clostridia.

Non-pathogenic (commensal) bacteria in the human gut play significant roles in the maintenance of health as well as in protecting the body against disease. For example, Huang et al (2005) showed that commensal microbiota influence steps in CD4 T cell differentiation moderating basal TCR signaling and immune responsiveness. Rao AV et al (2009) reported that, in patients with chronic fatigue syndrome, there was a significant decrease in anxiety symptoms (Beck Anxiety Inventories) in patients taking probiotics compared to controls (p=0.01).

Members of the clostridium species may be shown to play yet-to-be-elucidated roles in health and disease, for example, as mediators in the gut-brain connection, in the evolution of psychiatric illness (e.g. anxiety disorders), in the prevention of immune dysfunction and in acquired neurodevelopmental delay (e.g. autism and the autism spectrum disorders, or ASD). The basic fact remains, however, that most clostridia are non-pathogenic and are a “commensal” component of the gastrointestinal microbiota.

Some authors (e.g. Bolte E, 1998) believe that certain species of clostridium produce one or more toxins that may be factors in the development of some cases of autism. Interestingly, clostridia code for their toxins on genes located in plasmids; these short bits of DNA can be easily transferred from one organism to another and can thereby confer on a new organism the ability to make toxins where no such ability existed previously. Sandler et al (2000) treated 11 children with a diagnosis of regressive-onset autism, and who had a history of antimicrobial therapy, with vancomycin 500 mg/d for 8 weeks. Communication and behavior improved but, unfortunately, did not endure—two weeks after the trial ended, most had regressed and by 8 months all but one had returned to baseline levels.

Clostridium is an anerobic genus of bacteria and, as such, does not grow in the more aerobic environment found in the distal colon. However, all clostridia do produce endospores, many of which can survive for long periods under aerobic conditions. The new DDI Comprehensive Clostridium Culture uses special media and anaerobic culture conditions to isolate and identify over twenty clostridium species. Clostridia also produce specific toxins, two of which (C. difficile toxins A and B) are specifically analyzed at DDI.

There are approximately 100 different clostridium species, most of which are non-pathogenic (commensal) components of the human gut microbiota. Some authors (e.g. Bolte E, 1998) believe that certain species of clostridium produce one or more toxins that may be factors in the development of some cases of autism. Interestingly, clostridia code for their toxins on genes located in plasmids; these short bits of DNA can be easily transferred from one organism to another and can thereby confer on a new organism the ability to make toxins where no such ability existed previously. Sandler et al (2000) treated 11 children with a diagnosis of regressive-onset autism, and who had a history of antimicrobial therapy, with vancomycin 500 mg/d for 8 weeks. Communication and behavior improved but, unfortunately, did not endure—two weeks after the trial ended, most had regressed and by 8 months all but one had returned to baseline levels.

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![Clostridium Species Reported in Stool Analysis at DDI](image)

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benign inhabitants of the normal human gut, although a few are well-known pathogens. Clostridia are best known for their pathogenicity in disease entities such as food-borne illnesses, wound infections, pseudomembranous colitis and gas gangrene. The four main species that are pathogenic in humans are:
- *C. botulinum*—produces a toxin found in food and in wounds; causes botulism;
- *C. difficile*—produces seven types of neurotoxins (A-G) with most strains producing only one type but some strains may produce several toxins. Importantly, *C. botulinum* may transfer neurotoxin genes to other clostridia—this is of concern because methods designed to kill or inhibit *C. botulinum* may not always work well with other clostridium species;
- *C. difficile* can be a commensal organism in up to 5% of the population; long-term institutional care is an independent risk factor for colonization. Pathogenic *C. difficile* strains produce several toxins, the best known being toxins A and B, both of which cause diarrhea and inflammation and both of which can be tested for at DDI by using the “*C. difficile* toxins A and B” test as a stand-alone or as an add-on to other stool tests.
- *C. perfringens* (once called *C. welchii*) is ubiquitous in nature and is the third most common cause of food-borne illness in the US and UK. It also causes gas gangrene. When present in food it is very easily killed by heating to at least 74°C; the bad news is that the spores can withstand cooking temperatures and will germinate if cooked foods are left standing. Antibodies to the toxins of food-borne *C. perfringens* are common and most infections are sub-clinical.
- *C. tetani*—found in soil and wounds; produces toxins that causes tetanus (tetanus is the only vaccine-preventable illness that is infectious but not contagious from person to person).
- *C. botulinum* produces seven types of neurotoxins (A-G) with most strains producing only one type but some strains may produce several toxins. Importantly, *C. botulinum* may transfer neurotoxin genes to other clostridia—this is of concern because methods designed to kill or inhibit *C. botulinum* may not always work well with other clostridium species.

Non-pathogenic clostridium species now are reported in the “beneficial/expected” section of Doctor’s Data’s standard microbiology panel.
Behind the Scenes at DDI—Meet Dean Bass, Assistant Laboratory Director

Dr. Dean Bass’ career has focused on research and operations in the areas of atomic spectroscopy, including trace metal analysis, metal speciation, and neutron activation. He has used his expertise in a variety of projects to improve the analysis of hair, urine, blood and fecal testing for trace metals. He has developed a program to test water for the presence of toxic metals which supports and enhances doctors’ clinical findings related to metal toxicity. Dean also has been involved with the development of programs to test special samples, other than in blood, urine or stool. He has studied dental amalgams and the resultant release of mercury into the environment via feces. Dr. Bass’ current projects at DDI include improving laboratory accuracy and automation plus developing reference ranges for trace metals in animals. He has presented his findings in clinical journals and at numerous scientific meetings. In addition to his role at Doctor’s Data, Dean also is part of the research team at Argonne National Laboratory where his projects have been used to support the Department of Energy, the Environmental Protection Agency, the Army, the Navy, the Centers for Disease Control and prevention, and Homeland Security.

Dr. Bass’ undergraduate research at the University of Iowa looked at analyzing polyaromatic hydrocarbon compounds in coal using high performance liquid chromatography. He received his Master’s and Doctorate at the University of Texas at Austin studying trace metal analysis using thermal desorption mass spectrometry. As an applications scientist for Hitachi Instruments, he worked on trace metal analysis applications and contributed to the design of trace metal analysis instruments. He also teaches graduate and undergraduate level chemistry at the Illinois Institute of Technology. He has 26 publications and has given over 60 presentations.

What’s New at DDI—

by David Quig, PhD

DNA Oxidative Damage (urinary 8-hydroxy-2’deoxyguanosine)

DNA OXIDATIVE DAMAGE—Oxidation of DNA results in damage to guanosine residues and in the formation of 8-hydroxy-2’deoxyguanosine (8-OH-d-G). Elevated levels of 8-OH-d-G in urine provide a quantitative assessment of ongoing oxidative stress in the body. Chronic inflammatory processes and environmental factors such as toxic metals, chemicals, ionizing radiation; life style choices such as smoking and recreational drug use; and the use of some pharmaceuticals have been associated with increases in the hydroxyl radical that has been implicated in the oxidation of DNA. Enzymatic DNA repair mechanisms cut out the damaged residues which are then excreted in urine. Urinary 8-OH-d-G is a sensitive biomarker associated with many disease states including cancer (e.g. prostate), and chronic diseases (e.g. cystic fibrosis, atopic dermatitis, diabetes, rheumatoid arthritis and a wide variety of neurological conditions including Parkinson’s, Alzheimer’s and Huntington’s diseases). Moderately elevated levels of urinary 8-OH-d-G are associated with inadequate intake of carotenoids, antioxidant-rich foods and supplemental antioxidants.

Erythrocyte Glutathione (GSH)

ERYTHROCYTE GLUTATHIONE (GSH)—The level of total reduced and oxidized GSH in erythrocytes is a sensitive indicator of overall intracellular GSH status, cell viability and the ability of cells to withstand toxic challenges. GSH is important in many biological processes including detoxification of xenobiotics, removal of reactive oxygen species, regulation of the redox state of cells, the oxidative state of protein sulfhydryl groups and the regulation of immune function. Low levels of GSH have been reported in cardiovascular disease; cancer; AIDS; autism; alcoholism; neurodegenerative diseases; and chronic retention of toxic metals, chemicals and some pharmaceutical agents. Consequences of insufficient GSH include oxidative damage to proteins, membrane lipids and DNA.
Frequently Asked Questions

> On the DDI Comprehensive Stool Analysis please explain the clinical significance of finding “no growth” of yeast in the Mycology (Yeast) Culture section yet yeast cells are reported in the Microscopy section? . . . AS, Phoenix, AZ

Barb Berta MS, RD responds: Fairly often no yeast may be cultured yet we see yeast cells under microscopy in quantities of “few” to “many”. These cells could be dead organisms (e.g. coming from a food source) or they could be viable but very slow-growing and thus not showing up in the 3 to 5 day culture period. Either of these scenarios requires the clinician to ask more about diet and about symptoms that might relate to yeast overgrowth. A clue that yeast might be an issue, even though there is no evident growth, is a low level (2+ or less) of Lactobacillus or overall low levels of beneficial/expected bacteria. Typical symptoms of yeast overgrowth include headaches, sugar cravings, white coated tongue, diarrhea/constipation, rectal itching, bloating, abdominal pain and fatigue. ■

> I’ve heard that DDI is offering a new clostridium culture panel; what is this and how is it different from “Clostridium spp.” as reported under Bacteriology Culture and from the Clostridium difficile Toxins A and B test? . . . SZ, Miami, FL

Barb Berta MS, RD and Chuck Masur MD respond: Clostridia are common and expected commensal bacteria in almost everyone. The new Comprehensive Clostridium Culture reports all Clostridia species cultured from the stool specimen including potential pathogens such as C. botulinum, C. difficile, C. perfringens and C. tetani. If C. difficile were to be isolated on this panel, a C. diff Toxins A and B test would be done automatically and at no additional charge to the client. A report of Clostridium spp. under Bacteriology Culture simply indicates that one or more species of clostridia were cultured from a stool sample; it does not specify any particular organism (pathogenic or not). A clinician might “add on” a Clostridium difficile Toxins A and B test if a patient were to present with unexplained profuse diarrhea, with mucous and sometimes blood, which often is accompanied by fever and abdominal pain. Patients at risk for contracting C. difficile are those who have been on rounds of antibiotic therapy, in contact with animals, hospitalized, or visiting or working at a chronic care facility. ■

> Why does DDI always recommend ordering an unprovoked urine toxic metals test prior to an initial provoked urine test? . . . DD, Raleigh, NC

David Quig, PhD responds: The retention (body-burden) of toxic metals can be best estimated by comparing the levels of metals in urine before and after administration of a metal binding agent/chelator. The unprovoked specimen is important because it reveals current exposure to metals that are excreted by endogenous detoxification processes. The objective measure of metals currently being excreted permits calculation of the difference between the pre- and post-provocation specimens, the estimate of bioaccumulation of metals over time. For the most accurate estimation of metal retention the unprovoked urine specimen should be collected in very close temporal proximity to the provoked specimen. Without both unprovoked and provoked results, one is subject to a valid criticism that the provoked results might only reflect a recent acute exposure to metals. ■

We appreciate your continued interest and urge you to contact us should you have any questions. Our Customer Service Department is available between 8:00 and 6:00 CST Monday through Friday.