



LAB #: F\$\$\$\$\$!\$\$\$\$!  
PATIENT: GUa d'YDUjYbh  
ID: P\$\$\$\$\$\$  
SEX: Female  
AGE: 57

CLIENT #: %& ( )  
DOCTOR:  
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## Comprehensive Clostridium Culture; stool

### CLOSTRIDIUM CULTURE

#### Commensal Bacteria

1+ C. bifermentans  
1+ C. clostridioforme  
2+ C. hathewayi  
2+ C. limosum  
1+ C. perfringens  
2+ C. sordellii

#### Pathogenic Bacteria

1+ C. difficile

### INFORMATION

This test evaluates the presence and abundance of species of intestinal bacteria that are included in the *Clostridium* genus. The bacteria and their spores, derived from a stool specimen, have been cultured under very specific anaerobic conditions optimally suited for the growth of *Clostridium*. If non-pathogenic species are detected they are reported under the category of commensal and, their extent of growth in culture is reported as 1+ to 4+ (1+ being the least). If present in culture, the species that are well established to cause disease will be reported as pathogenic and be quantified as 1+ to 4+. If pathogenic *Clostridium difficile*, the most common cause of antibiotic associated diarrhea, is present in culture at any level additional testing will be performed to determine if the patient's bacteria are producing the specific toxins A and B (direct immunoassay on the cultured bacteria). If clinically significant species are identified a descriptive paragraph will be provided to facilitate interpretation of the test results.

Clostridia are anaerobic gram-positive bacteria that produce very durable spores as a means of proliferation; the spores are extremely resistance to antibiotics, heat, drying and disinfectants. When cultured under very specific anaerobic conditions optimized for growth of *Clostridium* species, the spores germinate to metabolically active bacteria that can be sub-cultured for positive identification (speciation).

*Clostridium* is a genus of bacteria that includes over one hundred distinct species, many of which are abundant and normal inhabitants (commensal) of the human gastrointestinal tract (GIT). Most of the *Clostridium* species are not virulent and can even have beneficial effects on health and integrity of the GIT in part by breakdown of polysaccharides and fermentation of carbohydrates to short chain fatty acids. However a few species are well-established opportunistic pathogens that produce specific toxins that cause diseases such as food-borne illnesses and, antibiotic-associated diarrhea and pseudomembranous colitis. Some species of *Clostridium* have been associated with neurological disorders and are the subject of ongoing research. Due to the biodiversity within the *Clostridium* genus it may be helpful to identify the prevalence of specific *Clostridium* species that are transiently or permanently present in the GIT of symptomatic patients.

#### References:

Handbook on Clostridia edited by P Durre. (2005) CRC Press  
Washington W, Allen S, Janda W et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6<sup>th</sup> edition. Lippincott Williams and Wilkins; 2006, pp. 931-939  
Clostridia. Louisiana Office of Public Health-Infectious Disease Epidemiology Section (2006)  
[www.infectiousdisease.dhh.louisiana.gov](http://www.infectiousdisease.dhh.louisiana.gov)  
Parracho HMRT, Bingham MO, Gibson G et al. Differences between the gut microflora of children with autism spectrum disorders and that of healthy children. J Med Microbiol (2005)54:987-91  
Song Y, Chengxu L and Finegold M. Real-time PCR quantitation of clostridia in feces of autistic children. Appl Environ Microbiol (2004)70:6459-65

Comments: Toxin testing performed for C. difficile, see C. difficile DNA report.

Date Collected: 10/29/2011  
Date Received: 11/2/2011  
Date Completed: 12/5/2011

v2.10

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## INTRODUCTION

This analysis of the stool specimen provides fundamental information about the overall gastrointestinal health of the patient. When abnormal microflora or significant aberrations in intestinal health markers are detected, specific interpretive paragraphs are presented. If no significant abnormalities are found, interpretive paragraphs are not presented.

### Clostridium difficile

*Clostridium difficile* (*C. difficile*) was detected in culture from this patient's stool specimen. *C. difficile* is a gram-positive anaerobic bacterium recognized as a major cause of antibiotic associated diarrhea and colitis. Infection is not uncommon in individuals who have taken broad spectrum antibiotics that eliminate beneficial/competing bacteria. Risk for infection is increased in individuals housed in places where *C. difficile* is prevalent (e.g. hospitals, chronic-care facilities). *C. difficile* is ubiquitous in nature and has been isolated from soil, sand, animal feces, and water. Approximately half of all healthy neonates carry *C. difficile* asymptomatically during their first year of life; the carrier rate decreases to about 3% in asymptomatic adults. *C. difficile* associated disease is not overtly expressed unless the bacteria actively produce toxins.

A molecular diagnostic assay utilizing DNA amplification technology which can detect all known strains of toxigenic *C. difficile* has been performed and the results of that test are provided separately. If the DNA test is negative for toxigenicity one need not be concerned; consider/evaluate the status of beneficial bacteria and resolve with probiotics if necessary. If the DNA test is positive for toxigenicity, symptoms can vary from mild, self-limited watery diarrhea that may subside with discontinuation of antibiotics, to severe persistent diarrhea and pseudomembranous colitis. Serious persistent symptoms in patients with *C. difficile*-associated disease may warrant antibiotic treatment with oral vancomycin or metronidazole. Relapse of *C. difficile*-associated disease sometimes occurs with antibiotic therapy. Cessation of the initial antibiotic and change to an agent less likely to cause diarrhea may be warranted.

Washington W, Allen S, Janda W et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th edition. Lippincott Williams and Wilkins; 2006.

Murray PR, Baron EJ, Jorgensen JH et al. *Manual of Clinical Microbiology*, 9th edition. ASM Press, Washington DC;2007.

Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA).

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### Clostridium Species and Autism

One or more of the Clostridium species that have been postulated to have a role in some of the symptoms of autism were cultured from this patient's stool specimen. The Clostridium species of current research interests include C. bolteae, the C. hystolyticum group (includes C. hystolyticum, C. limosum and C. proteolyticum), C. botulinum and C. tetani. If the well-established pathogenic species C. botulinum or C. tetani were found, separate interpretive paragraphs have been provided in this report.

A hypothesis was presented that repeated antibiotic use in children might cause bacterial imbalance in the gastrointestinal tract (GIT) such that beneficial and, nonpathogenic commensal bacteria have been consequently diminished and replaced by one or more toxin producing species (Bolte, 1998). Clostridium species that produce neurotoxins and potentially toxic metabolic byproducts have been reported to be more prevalent in autistic children compared to neurotypical controls; most notable were greater quantities C. bolteae and members of the C. hystolyticum group. Support for a connection between toxin producing bacteria in the GIT and autistic behavioral abnormalities and gastrointestinal symptoms was provided from a study in which a select sub-group of "regressive-onset" autistic children exhibited transient yet significant improvements while taking a minimally absorbed antibiotic. However the gains attained were lost about two weeks after cessation of antimicrobial therapy. The rebound in symptoms may relate to the fact that the antibiotic does not kill the spores produced by Clostridium species and recolonization by the toxin-producing bacteria likely occurred. The treatment protocol utilized is not recommended as useful therapy but supports the gut flora-brain connection and provides incentive for further research, particularly with focus on ways to eradicate clostridial spores and improve GIT flora.

The aforementioned Clostridium species produce not only species-specific neurotoxins but also toxic metabolic byproducts. By proteolytic fermentation they produce metabolites in the GIT such as ammonia, amines, volatile phenols, and indoles, which are toxic and present at only low levels in the normal GIT. The amines produced by proteolytic Clostridium species include histamine, cadaverene, thymine and putrescene, which are pharmacologically active and can affect a variety of physiological functions.

Bolte ER. Autism and Clostridium tetani. Medical Hypotheses (1998)51:133-44.  
Sandler RH, Finegold SM, Bolte ER et al. Short-term benefit from oral vancomycin treatment of regressive-onset autism. J Child Neurol (2000)15:429-35.

Song Y, Liu C and Finegold M. Real-time PCR quantitation of Clostridia in feces of autistic children. Appl Environ Microbiol (2004)70:6459-65.

Parracho HMRT, Bingham MO, Gibson GR et al. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol (2005)54:987-91.

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**Clostridium Culture**

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