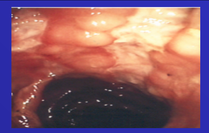

# Evaluation of Fecal Biomarkers for Monitoring Antibiotic Treatment of a Patient Infected with *Clostridium difficile*

R. J. Carman[1], J. H. Boone[1], R. Albert[1], H. Grover[2], K. N. Wickham[1], and D.M. Lyerly[1]

[1]TECHLAB®, Inc., Blacksburg, Virginia and New River Gastroenterology[2], Christiansburg, Virginia

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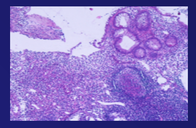


## ABSTRACT

**Background: A 66 year old male developed *C. difficile* infection (CDI) following antibiotic treatment for oral infection. After treatment failure with metronidazole, the patient (pt) began vancomycin followed by 1 month of rifaximin and symptoms resolved. One month following rifaximin treatment, the patient suffered a clinical relapsed with diarrhea and abdominal pain and began treatment with Dificid®. Aim: Our study evaluated fecal biomarkers for response to treatment using serial stool sampling. Methods: A total of 33 stool samples were collected from a pt with CDI before, during and after antibiotic treatment. Toxin (optical density), glutamate dehydrogenase (GDH; ng/g), and lactoferrin (Lf; normal <7µg/g) were determined in feces with in vitro diagnostic assays. *C. difficile* isolated by ethanol-shock culture was then ribotyped by PCR. Results: During the onset of symptoms, toxin was positive, GDH and Lf levels were very high (6980 ng/g and 6784 µg/g, respectively), and toxigenic ribotype ARL056 was isolated. Dificid® was begun and by day 4 of treatment, stool toxin was negative, GDH was low (20 ng/g), Lf decreased to 44µg/g, and symptoms had resolved. On day 6, GDH and toxin were undetectable, Lf was normal and culture was negative. The pt remained *C. difficile*-negative for 10 days post treatment then became GDH, culture and stool toxin positive with the same ribotype (ARL056) but without symptoms and with normal Lf. The pt remained *C. difficile* positive by culture and by GDH for 3 weeks before becoming negative. Conclusions: Lf, GDH and toxin correlated with CDI and became negative by day 6 of treatment with Dificid® indicating a response to treatment. At 10 days post treatment, *C. difficile* returned and spontaneously resolved. These are the first results to show the potential of monitoring GDH toxin and Lf levels, as indicators of effective treatment for CDI patients.**

## INTRODUCTION

**The current epidemic of *C. difficile* diarrhea affects most often the elderly, the already ill and those in hospitals. Exposure to antibiotics is an important risk factor. After diagnosis the progression from treatment, with ironically yet further antibiotics, to recovery can be straightforward or it may be complicated by the reappearance of symptoms. Since about one in five patients has a symptomatic relapse and as serial relapses can occur, relapses are potentially expensive as well as devastating to the individuals affected. The speedy identification of treated patients at risk of relapse would allow physicians an earlier and possibly wider choice of treatments, ranging from observation and stopping the antibiotic that produced the condition in the first instance, to the use of perhaps vancomycin over metronidazole, or fidaxomicin over both, conserving the effectiveness of these important drugs.**

## AIM

**To evaluate fecal biomarkers for response to treatment using serial stool samples and a standard ribotype library.**

**Robert J. Carman e-mail: rjcarman@techlab.com**

## METHODS

**Patient history - A 66 year old male developed *C. difficile* infection (CDI) following antibiotic treatment for oral infection. After treatment failure with metronidazole, the patient began vancomycin followed by 1 month of rifaximin and symptoms resolved. One month following rifaximin treatment, the patient suffered a clinical relapsed with diarrhea and abdominal pain and began treatment with Dificid®.**

**Stool specimens - A total of 33 stool samples were collected from a patient with CDI before, during and after antibiotic treatment. The study was approved by TECHLAB's IRB and the patient gave his informed consent to the study. The results did not form part of the patient's laboratory diagnosis. Bristol Stool Chart was used to report consistency.**

**Glutamate dehydrogenase (GDH) was determined by the TECHLAB *C. DIFF CHEK* ™ -60 test according to the Package Insert and quantitatively (ng/mL) using a modified TECHLAB *C. DIFF CHEK*™ -60.**

**Toxins A & B were determined using the TECHLAB *C. DIFFICILE TOX-B TEST* (tissue culture) and *C. DIFFICILE TOX A/B II*™ tests as instructed by the Package Inserts and results were reported as Yes/No and with optical densities at 450/620nm (Dual OD), respectively.**

**Fecal lactoferrin was measured quantitatively using the TECHLAB *IBD - SCAN*® as instructed by the Package Insert and results are reported as µg/mL stool.**

**Bacterial culture: Ethanol spore selection with CCFA was used to identify culture-positive specimens. Isolates were subcultured to BHI and grown for 72h.**

**Ribotyping analysis: DNA was extracted from broth cultures using the QIAamp Mini Kit (Qiagen, Valencia, CA) and amplified. Banding patterns were compared to a standard library.**

## RESULTS

**Table 1. Results during longitudinal analysis.**
**•As can be seen, analyte concentrations fell on initiation of Difficid treatment.**
**•Two weeks after treatment there was an asymptomatic, same-strain relapse.**
**• Although *C. difficile* (ribotype 056) was grown and the pt's stools also contained GDH and toxins, there was no diarrhea and lactoferrin levels remained normal (<7µg/g).**

| Date | Bristol stool | Abdominal pain | ABII Dual OD | Tissue culture | Lactoferrin µg/mL | Treatment | CHEK-60 GDH OD | GDH SCAN ng/mL | CCFA culture |
|---|---|---|---|---|---|---|---|---|---|
| 9/23 | 7 | Yes | 2.161 | Pos | 6784 | No | 4.161 | 6980 | ND |
| 9/24 | 2 | Yes | 3.749 | Pos | 73 | No | 3.953 | 1310 | Pos |
| 9/26 | 2 | No | 1.099 | Pos | 65 | No | 3.964 | 3310 | Pos |
| 9/28 | 2 | No | 1.369 | Pos | 2 | No | 3.445 | 1900 | Pos |
| 9/30 | 2 | No | 3.036 | Pos | 7 | Dificid 8pm | 4.067 | 1040 | ND |
| 10/1 | 2,mucus | No | 3.334 | Pos | 11 | Dificid | 4.162 | 4100 | ND |
| 10/2 | 2,mucus | No | 3.578 | Pos | 32 | Dificid | 4.082 | 2700 | ND |
| 10/3 | 2 | No | 0.003 | Pos | 42 | Dificid | 1.885 | 60 | ND |
| 10/4 | 2 | No | 0.003 | Neg | 44 | Dificid | 0.821 | 20 | Neg |
| 10/5 | 2 | No | 0.002 | Neg | 19 | Dificid | 0.189 | Not done | Neg |
| 10/6 | 2 | No | 0.003 | Neg | 1 | Dificid | 0.001 | 0 | Neg |
| 10/7 | 2 | No | 0.003 | Neg | 3 | Dificid | 0.002 | 0 | Neg |
| 10/8 | 2 | No | 0.002 | Neg | 7 | Dificid | 0.003 | 0 | Neg |
| 10/9 | 2 | No | 0.002 | Neg | 2 | Dificid | 0.003 | 0 | Neg |
| 10/10 | 2 | No | 0.003 | Neg | 2 | Dificid | 0.003 | 0 | Neg |
| 10/11 | 2 | No | 0.001 | Neg | 1 | No | 0.005 | 0 | Neg |
| 10/12 | 2 | No | 0.002 | Neg | 4 | No | 0.003 | 0 | Neg |
| 10/13 | 2 | No | 0.005 | Neg | 4 | No | 0.005 | 0 | Neg |
| 10/14 | 2 | No | 0.003 | Neg | 2 | No | 0.002 | 0 | Neg |
| 10/18 | 2 | No | 0.003 | Neg | 1 | No | 0.002 | 0 | Pos |
| 10/23 | 2 | No | 0.004 | Pos | 1 | No | 0.003 | 0 | Pos |
| 10/26 | 2 | No | 0.308 | Pos | 1 | No | 0.502 | 130 | Pos |
| 10/30 | 2 | No | 0.004 | Pos | 2 | No | 2.659 | 190 | Pos |
| 11/3 | 2 | No | 0.064 | Pos | 1 | No | 1.785 | 130 | Pos |
| 11/10 | 2 | No | 0.038 | Pos | 1 | No | 2.413 | 680 | Pos |
| 11/16 | 2 | No | 0.005 | Pos | 0 | No | 0.068 | 0 | Pos |
| 11/23 | 2 | No | 0.002 | Neg | 1 | No | 0.002 | 0 | Neg |
| 12/3 | 2 | No | 0.003 | Neg | 1 | No | 0.001 | 0 | Neg |

## NOTES ON COURSE OF DISEASE

**•Prior to Sept 23: Earlier CDI responded to rifaximin**

**•Sept 23: Symptomatic relapse: Pain, then diarrhea; Stool contained ribotype 056 spores, GDH, toxin & elevated lactoferrin**

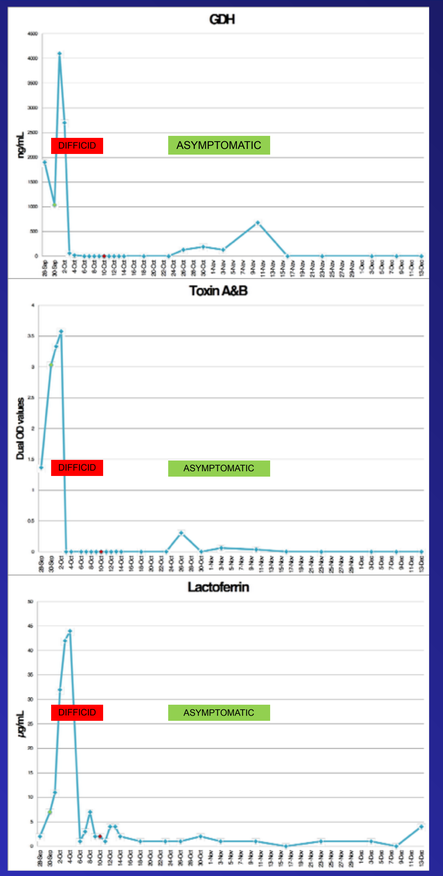
**•Sept 30 to Oct 10: Difficid**

**•Oct 1: Symptoms resolved, did not return**

**•Oct 4: Stool toxin negative, GDH low (20 ng/g), Lf decreased (44µg/g)**

**•Oct 6: GDH & toxin undetectable, Lf normal, culture negative.**

**•Oct 23: Pt asymptomatic with normal Lf; became GDH, culture (ribotype 056) & stool toxin positive. GDH & culture positive for 3 weeks until going negative.**




## CONCLUSIONS

**•Monitoring GDH, toxins and lactoferrin are useful as indicators for effectiveness of treatment in patients with CDI.**

**•Identifying a response to treatment may improve patient outcomes and result in better antibiotic stewardship.**

**•Future studies are needed to determine if monitoring response to treatment can predict the risk for relapse.**