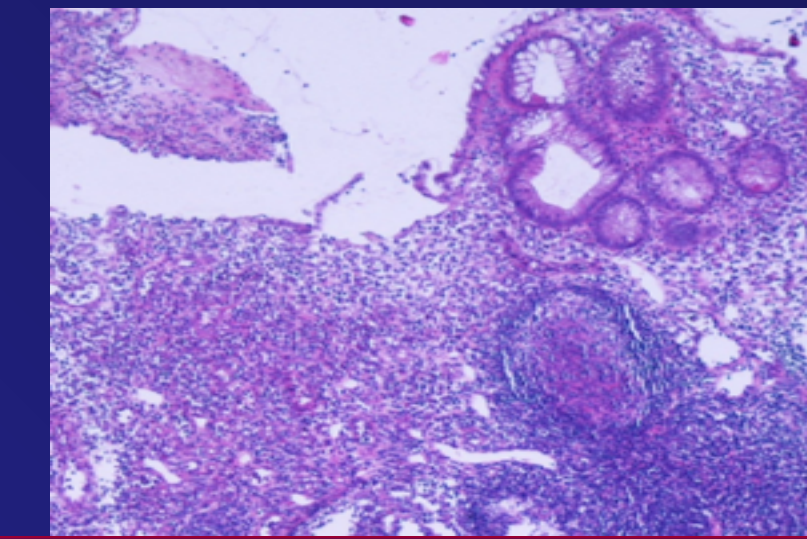# Lactoferrin as a Predictor of Disease Severity for *Clostridium difficile* Disease

J. H. Boone[1], C. McCoy[1], J. Gotthardt[1], H. Totty[1], J. DiPersio[2], M. Tan[2], S.J. Salstrom[2] and D.M. Lyerly[1]

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INTRODUCTION

Clostridium difficile infection (CDI) involves a range of clinical presentations from self-limiting diarrhea to life-threatening pseudomembranous colitis and mega colon. Patients usually experience at least 3 watery stools without blood per day and may have abdominal pain or cramping. Currently, a combination of clinical presentations and various laboratory parameters have been proposed for stratifying patients from mild to severe disease. White blood cell (WBC) count, serum albumin and creatinine levels are the most commonly used lab indicators for disease activity of CDI. While most episodes resolve with treatment, up to 25% of these cases relapse and require a second round or more of antibiotics. An initial episode of mild CDI is often treated with metronidazole, while vancomycin is saved for moderate to severe or relapsing cases. 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.

AIM

We measured fecal lactoferrin in patients with clinically defined CDI (mild to severe) to investigate the link between elevated levels of lactoferrin and disease severity.

METHODS

- 39 patients suspected of having *C. difficile* disease based on clinical history of prior antibiotic use, more than 3 unformed stools per day and the presence of fecal glutamate dehydrogenase (GDH) were enrolled over a 6 month period with informed consent. A total of 53 healthy subjects (no intestinal illness) were included as controls (normal lactoferrin range = 1.45±0.4 µg/mL).

- Disease severity: Mild = diarrhea no other symptoms, Moderate = diarrhea with some symptoms of pain, fever, vomiting, elevated WBC count, nausea, comorbidities, Severe = diarrhea and most symptoms listed.

- Tissue culture: Human foreskin cell monolayers and toxin B neutralizing sera were used for specific neutralization with feces and cultures.

- Bacterial and toxigenic culture: Ethanol spore enrichment with CCFA was used to identify culture-positive specimens. Isolates were subcultured to BHI and grown for 72h then tested by tissue culture for the presence of toxin B.

- PCR ribotyping analysis: DNA was extracted from broth cultures using the QIAamp Mini Kit (Qiagen, Valencia, CA). Control CD strains (ARL ribotypes 001, 002, 003, 012, 014, 017, 027, 033, 036, 046, 053, 054, 078, 106, 110, 126 and 154) were used for ribotyping standards.

- *C. DIFF CHEK-60*™ test: This test is a microwell ELISA that provides results for the presence of antigen (GDH).

- *IBD-SCAN*®: quantitative ELISA for measuring stool lactoferrin concentration (µg/mL).

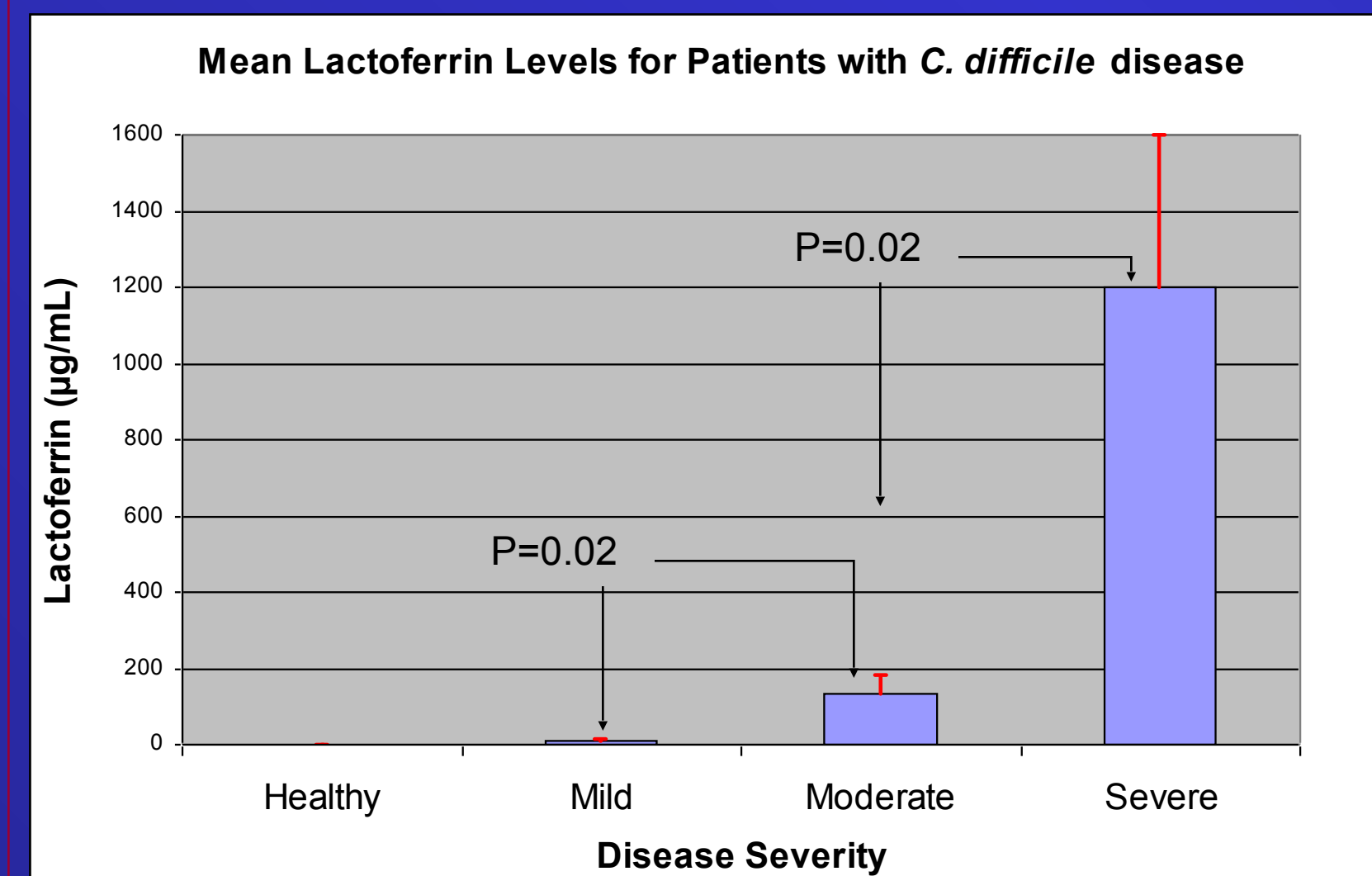- Statistical analysis: Student T-test for p-value, mean±standard error by Microsoft Excel.

RESULTS

Table 1. Patient Characteristics

Patient Characteristics		Percent of Total N=39	Percent of Severe N=15	Percent of Moderate N=21	Percent of Mild N=3
Gender	Male	41	60	29	33
	Female	59	40	71	67
Age	< 65 yr	44	40	48	33
	>64 yr	56	60	52	67
Pain	Yes	67	60	71	67
	No	33	40	29	33
Co-morbidities	Diabetes	31	13	29	33
	Cancer	23	13	29	33
	Renal failure	23	20	29	0
	Pneumonia	18	27	10	33
Stool Consistency	Solid	3	0	5	0
	Semi-solid	44	33	43	100
	Liquid	54	67	52	0
Clinical Assessment	Severe	38	100	0	0
	Moderate	54	0	100	0
	Mild	8	0	0	100

Summary for Table 1: Most patients were > 64 yr, experienced pain, had liquid stools. Diabetes, Cancer and renal failure were the top 3 co-morbidities.

Table 2. *C. difficile* Detection Rates for Stratified Patient Populations

Patient Group	Percent Toxigenic Culture Pos.	Percent CHEK-60 GDH Pos.	Percent Tissue Culture Pos.
Severe	93%	93%	87%
Moderate	86%	100%	71%
Mild	100%	100%	33%
ARL 027	100%	100%	91%
Non 027	82%	94%	53%

Summary for Table 2: Detection for fecal GDH compared similarly to bacterial culture. There was a specimen with no growth and a single specimen that was GDH-negative when repeated. Tissue culture positive rate for toxin B was lowest for the mild and non027 groups.

Figure 1. Lactoferrin Levels by Disease Severity

Mean Lactoferrin Levels for Patients with *C. difficile* disease
P=0.02
P=0.02
(Lactoferrin µg/mL; Healthy, Mild, Moderate, Severe; Disease Severity)

Summary for Fig. 1: Lactoferrin levels were significantly higher between mild, moderate and severe groups and trended higher with more severe disease.

Figure 2. WBC Counts by Disease Severity

Percent Elevated WBC Counts
P=0.08
(Percent (%); Mild, Moderate, Severe; Disease Severity)

Figure 3. 027 Isolation by Disease Severity

Percent ARL 027 Isolates
(Percent (%); Mild, Moderate, Severe; Disease Severity)

Summary for Figs 2 and 3: WBC counts trended higher (not significant) from mild to severe disease. Severe and Moderate groups comprised mostly ARL 027 ribotype isolates.
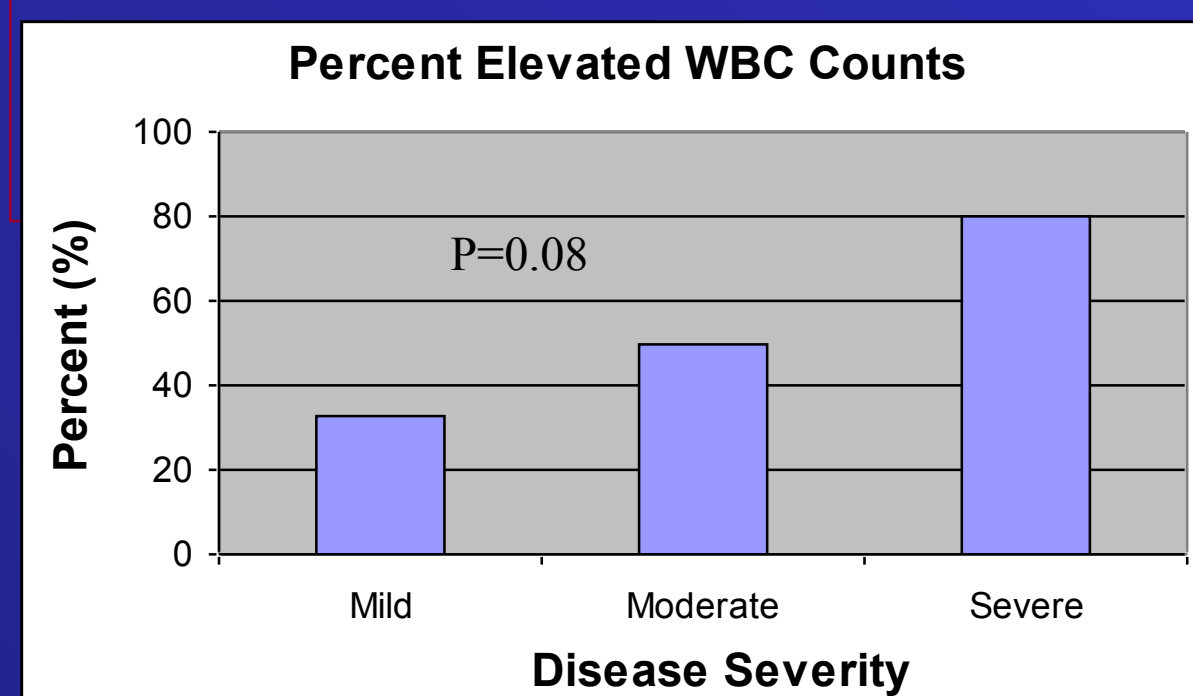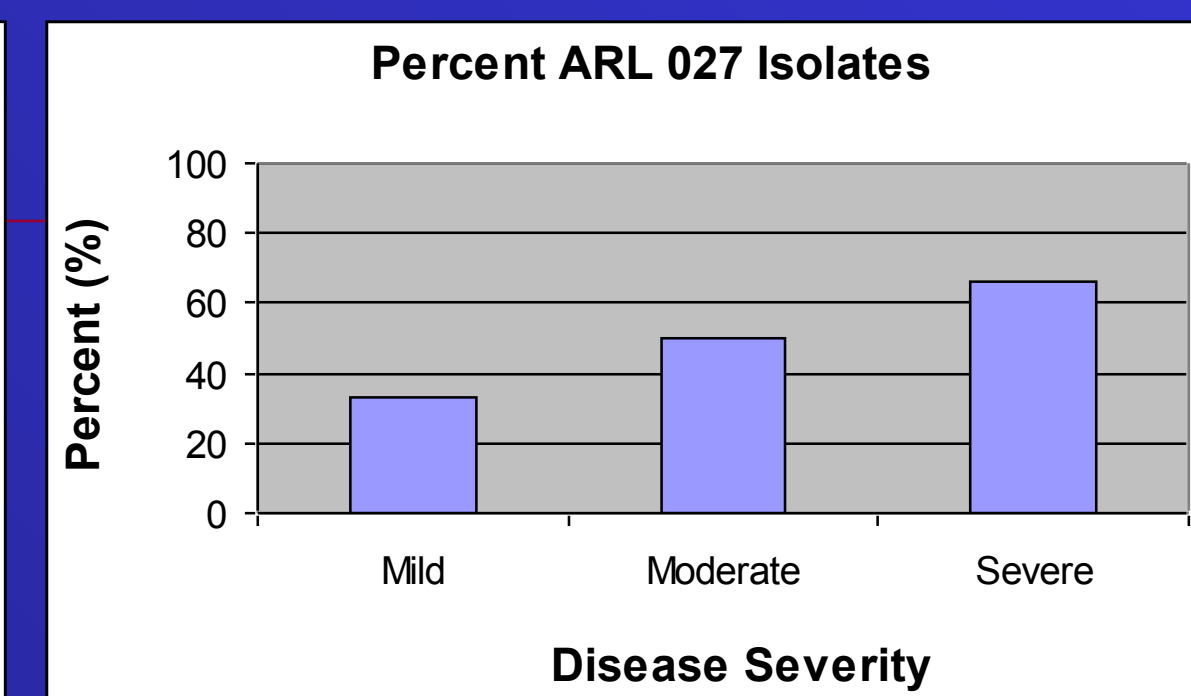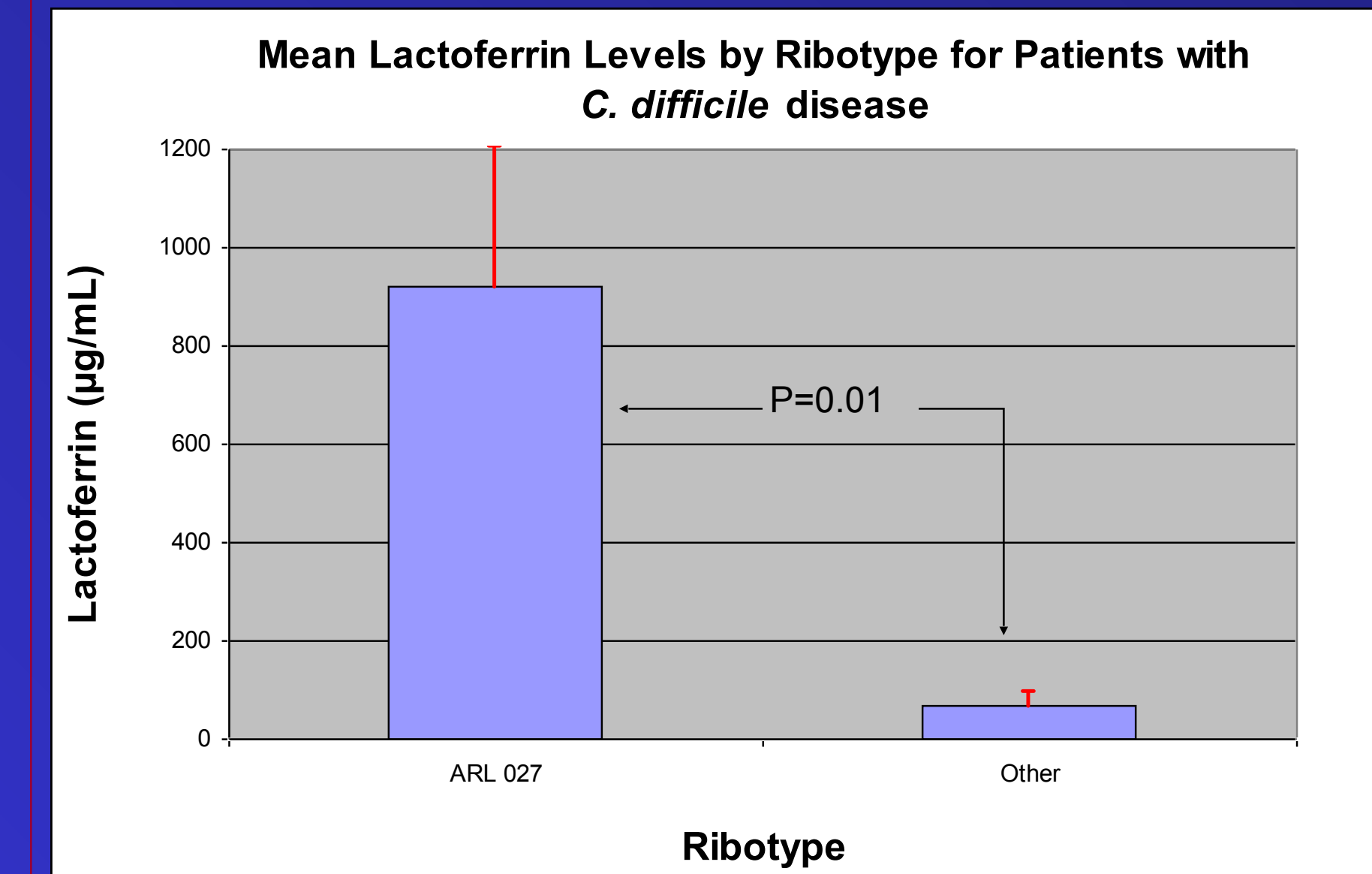
Figure 4. Lactoferrin Level by Ribotype

Mean Lactoferrin Levels by Ribotype for Patients with *C. difficile* disease
P=0.01
(Lactoferrin (µg/mL); ARL 027, Other; Ribotype)

Summary for Fig. 4: Lactoferrin levels were significantly higher for the ARL 027 vs. non027 group. The isolation rate for 027 was 57%.

CONCLUSIONS

- Elevated levels of lactoferrin are useful as an aid for stratifying patients based on disease severity.

- Ribotype ARL 027 is associated with higher lactoferrin levels and more severe disease as determined by clinical assessment.

- Toxigenic culture and the presence of fecal GDH performed similarly in detecting ARL 027 and non027 *C. difficile*.

References cited

1. Pant *et al*. 2009. Laboratory predictors of mortality in patients with *C. difficile*. J Investigative Med. 57:1-3.
2. Henrich *et al*. 2009. Clinical Risk Factors for Severe *C.difficile*-associated disease. Emerg. Infect. Dis. 15:415-421.

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