Scientific Symposium on New Approaches to *Clostridium difficile* Testing

Conference report from the Satellite Symposium held during the 2010 United European Gastroenterology Week (UEGW)

23rd–27th October
Barcelona, Spain

Chairwoman:
Dr Beryl Oppenheim (Birmingham, UK)
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A satellite symposium held during the 18th United European Gastroenterology Week (UEGW) of the United European Gastroenterology Federation (UEGF) in Barcelona, Spain, addressed new approaches to *Clostridium difficile* testing. The symposium was chaired by Dr Beryl Oppenheim of the Sandwell and West Birmingham Hospitals NHS Trust, UK.

Dr Oppenheim welcomed attendees and provided an introduction to the dilemmas facing clinicians treating patients with possible *C. difficile* infection, despite tests for the infection being available for many years. There continues to be multiple clinical challenges to question with this patient population, such as: Is this a single case? What is the best diagnostic test to use? Is there a possibility of false-positive or false-negative diagnostic tests? Is this a first infection or a recurrence? How severe is the infection likely to be? What is the best treatment plan for this patient and individuals close to them?

These questions were discussed in the context of the current and future management of *C. difficile* infection with presentations by experts from Europe and North America. Prof. Tracy Wilkins, Professor Emeritus at Virginia Tech and Co-founder and Chairman of the Board of TechLab®, Blacksburg, USA, discussed the history of *C. difficile* research and the development of commercial assays to detect pathogenic toxins. Prof. Michel Delméé, head of microbiology at the Brussels Saint-Luc University Hospital in Belgium, compared the relative benefits of commercial detection methods and provided a two-step algorithm for diagnosing *C. difficile* infection. Finally, Prof. Jost Langhorst, of the Department of Internal and Integrative Medicine at the University of Duisburg-Essen, Germany, reviewed the role of lactoferrin as a biomarker for inflammation in both *C. difficile* infection and inflammatory bowel disease (IBD). A summary of these presentations follows below.

**C. difficile** testing: past, present, and future

Identifying the cause of pseudomembranous enterocolitis

Prof. Tracy Wilkins provided an overview of the discovery of *C. difficile* disease and the evolution of diagnostics for detecting the pathogen and its toxin. New ideas for future diagnostic aids were discussed, including fecal lactoferrin as a biomarker for inflammation and a new evidence-based protocol utilizing glutamate dehydrogenase (GDH) as a method for eliminating specimens that are negative for *C. difficile*. Interest in the management and assessment of *C. difficile* infection has risen in the past few years, most recently with the increased incidence of *C. difficile* disease and the introduction of new detection methods. Today, the incidence of *C. difficile* disease continues to rise and involves both nosocomial and community acquired infections.

The history of *C. difficile* disease began with the discovery in the early 1970s of severe pseudomembranous enterocolitis associated with clindamycin antibiotic therapy. More research into antibiotic-related colitis followed, and *C. difficile* was eventually identified as the cause. A pivotal report in 1976 of 16 cases of severe colitis, in which four patients died, highlighted the risk of severe complications, and the need for a specific treatment for the disease.

Although pseudomembranous enterocolitis was recognized as a deadly condition, its cause was not determined until years later. Clinicians ruled out allergic reaction or direct toxicity of the antibiotic received. Researchers identified a cytotoxic effect on tissue culture cells that was not due to a virus and that could not be propagated. While no pathogenic bacteria, *Mycoplasma* or viruses were found, it was suspected that pseudomembranous colitis was caused by a bacterial toxin and the hunt accelerated to unravel the mystery.

Progress for identifying the pathogen was made when it was discovered that *C. sordellii* antitoxin neutralized the effect of the cytotoxin. Although *C. sordellii* was not present in these samples, *C. difficile*, which produces a similar cytotoxin, was isolated from samples of patients with pseudomembranous colitis and subsequently identified as a potential pathogen (Table 1).

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Research team</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>Undescribed toxin in pseudomembranous colitis</td>
<td>Larson et al.</td>
</tr>
<tr>
<td>1977</td>
<td>Antibiotic-induced colitis implications of a toxin neutralized by <em>C. sordelli</em> antitoxin</td>
<td>Rifkin et al.</td>
</tr>
<tr>
<td>1977</td>
<td>Clindamycin-associated colitis due to a toxin-producing species of <em>Clostridium</em> in hamsters</td>
<td>Bartlett et al.</td>
</tr>
<tr>
<td>1978</td>
<td>Identification of <em>C. difficile</em> as a cause of pseudomembranous colitis</td>
<td>Multiple research teams</td>
</tr>
</tbody>
</table>

Initially, *C. difficile* was not thought to be responsible for the colitis; it was listed as non-pathogenic because it was a component of the normal fecal flora in about half of all newborn infants. The reasons why the presence of *C. difficile* is not harmful to infants remains unknown, but in adults, the organism can cause pseudomembranous colitis. Wilkins and colleagues successfully produced an antitoxin to *C. difficile*.

**Developing a C. difficile toxin assay**

It was later determined that *C. difficile* produced a second toxin that could be separated from the cytotoxin by ion-exchange chromatography. These findings were subsequently confirmed by Wilkins and colleagues, and both groups of researchers named the Toxins ‘A’ and ‘B’, representing the enterotoxic and cytotoxic toxins, respectively.

The enzyme immunoassay (EIA) for detection of Toxin A was developed by Wilkins and colleagues early after the discovery of the connection between Toxin A and colitis. Shortly afterwards, another research group claimed that a latex test, which was already available at that time, also detected Toxin A. In 1986, Wilkins and Lyerly determined that the commercial latex test for Toxin A did not, in fact, detect the toxin, but instead, detected GDH, an essential metabolic enzyme that is produced by both toxigenic and non-toxigenic strains. The detection of fecal GDH turned out to be a sensitive indicator for the presence of *C. difficile*. The *DIFF QUIK CHEK COMPLETE®* (TechLab®) is a newly developed diagnostic aid that utilizes the detection of both GDH and Toxins A and B. Because GDH is produced by other bacteria, a

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monoclonal antibody is necessary to ensure specificity. The resulting assay has a sensitivity of 85–90% compared with toxigenic culture.

Non-toxigenic *C. difficile* strains will react in the GDH test and can occur in 5–20% of antibiotic-associated diarrhea (AAD), depending on the institution conducting the testing. At Prof. Wilkins’ institution in Virginia, approximately 11% of strains identified by the test are non-toxigenic (Wilkins, unpublished data). By comparison, very few strains identified in nursing homes are non-toxigenic. However, a much higher percentage of strains identified in young people are non-toxigenic *C. difficile*.

The greatest utility of the *C. diff Quik Chek Complete®* is that it can be used prior to real-time polymerase chain reaction (PCR) as a rapid and inexpensive screening test. Results can be obtained within 30 minutes. Real-time PCR emerged in 2009 as a method to detect rapid and inexpensive screening test. Results can be obtained within can be used prior to real-time polymerase chain reaction (PCR) as a screening with the *Quik Chek Complete®* and then confirming the diagnosis with real-time PCR only in cases that are neither clearly positive nor negative for GDH and toxin.

Interpreting results

Does a positive *C. difficile* test indicate disease? *C. difficile* is often found in younger patients without causing disease. In fact, Prof. Wilkins noted that we should remember that babies often harbor *C. difficile* in the absence of disease. There have been reports of *C. difficile*-associated colitis in younger patients, but younger patients are generally much more resistant to the disease.1,16 It is unclear why younger people tolerate the presence of *C. difficile* without developing colitis, but an intact colonic mucosa and a better immune system may play a role.

*C. difficile* disease is an inflammatory condition with an appearance similar to that of ulcerative colitis (UC), with respect to the influx of activated neutrophils in the colonic mucosa (Figure 1).

“This is an inflammatory disease,” noted Prof. Wilkins. “The appearance of small areas blossoming into little volcanoes of eruption of neutrophils coming into the colon is characteristic of moderate to severe *C. difficile* disease”. Tests to detect this increase in neutrophils allow clinicians to determine if patients are presenting with an inflammatory illness. This inflammatory response, with associated tissue necrosis, is the primary pathogenic element of pseudomembranous enterocolitis in severe cases, rather than simply the presence of the *C. difficile* organism or toxins.

Lactoferrin assays for intestinal inflammation

Lactoferrin is a protein found in high concentrations in activated neutrophils that infiltrate the intestinal lumen and serve as a monitor for intestinal inflammation. Several lactoferrin assays are available for detecting increased levels in feces. The TechLab® lateral flow tests *LEUKO EZ VUE™* and *IBD EZ VUE*® are rapid and inexpensive, and easy to use. The *Leuko EZ Vue™* detects fecal white blood cells in acute cases of diarrhea (infectious) and the *IBD EZ Vue®* is more focused on the differentiation of active IBD from irritable bowel syndrome (IBS). The MicroWell® ELISA tests, *IBD-CHEK®* and *IBD-SCAN®*, are qualitative and quantitative tests, respectively. These tests are useful when a large number of patient specimens must be examined. The *IBD-Chek®* differentiates active IBD from IBS and healthy persons, and *IBD-Scan®* offers a quantitative result as an indicator of the amount of inflammation in active IBD.

*IBD-Scan®* may also be useful for gauging *C. difficile* disease severity. Recent data show a direct correlation between the severity of disease in *C. difficile* infection as assessed by clinicians and the level of lactoferrin (Table 2). These results show an approximately 10-fold differential between patient groups. Such a clear difference in lactoferrin levels can aid clinicians who currently determine severity based on symptoms, such as abdominal pain and number of stools per day.

Table 2 – Lactoferrin level in patients with clinically defined *C. difficile* infection

<table>
<thead>
<tr>
<th>C. difficile disease activity</th>
<th>N</th>
<th>Mean lactoferrin level (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>13</td>
<td>1,350</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>160</td>
</tr>
<tr>
<td>Mild</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Healthy</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

Recently, a simple laboratory algorithm for diagnosing *C. difficile* infection was proposed.17,18 A screening algorithm with *C. diff Quik Chek Complete®,* which allows for simultaneous detection of both GDH and Toxins A and B, followed by additional testing with the lactoferrin assay, PCR, or toxigenic culture as needed, was developed and tested (Figure 2). Approximately 80% of samples are determined within 30 minutes to be negative by *C. diff Quik Chek Complete®,* as indicated by the absence of GDH or toxin. Another 10% are positive for both GDH and toxin, and are therefore
conclusively positive for *C. difficile* infection. The remaining 10% of samples are positive for GDH but negative for toxin, and therefore require further testing. This result can indicate the presence of a non-toxigenic strain of *C. difficile* or a toxigenic strain that has not yet produced enough toxin to test positive.

Using the proposed algorithm, samples requiring further testing for *C. difficile* diagnosis can be evaluated for inflammation using the level of lactoferrin or can be tested with PCR or toxigenic culture. Testing for elevated lactoferrin offers an inexpensive, rapid option to detect inflammation. Patients negative for lactoferrin (inflammation) can avoid unnecessary treatment with antibiotics, which itself can cause *C. difficile* infection.

Relapse is common in patients and occurs in about 25% of cases, particularly in those treated with the antibiotics metronidazole or vancomycin. A subsequent infection may involve a more virulent strain or more severe infection. Recently, a new strain of *C. difficile*, ribotype 027, was identified that is associated with an increased infection-related mortality rate. This virulent strain has been reported across Europe and North America and is a predominant ribotype. The appearance of this new strain may be largely responsible for the increase in *C. difficile* infection observed over the last decade in Europe as well as in North America.

Clinicians now have multiple diagnostic tests for *C. difficile* and must determine which strategy is most appropriate for their patient. Effective management also depends on identifying which patients who test positive for *C. difficile* actually require treatment. Examining a fecal sample for *C. difficile* toxin alone may not be a reliable diagnostic parameter. With its simultaneous detection of *C. difficile* antigen (GDH) and Toxins A and B, the *C. diff Quik Chek Complete* is a rapid and reliable test that, when coupled with a lactoferrin test, offers a more complete clinical picture to enable effective patient management. Additionally, assessing the lactoferrin level can help physicians identify rapidly and accurately those patients who are exhibiting an inflammatory response and may therefore require intervention, and those who are tolerating the infection well and can therefore avoid unnecessary treatment.

With its simultaneous detection of *C. difficile* antigen (GDH) and Toxins A and B, the *C. diff Quik Chek Complete* is a rapid and reliable test that, when coupled with a lactoferrin test, offers a more complete clinical picture to enable effective patient management.

Comparing *C. difficile* antigen and toxin testing with bacterial culture

Prof. Michel Delmée discussed the relative benefits of different *C. difficile* testing methods and reviewed a current two-step algorithm for *C. difficile* diagnosis in the clinical setting. Over the last several years there have been important improvements in the laboratory diagnosis of *C. difficile* infections. Clinicians now have much better diagnostic tools and are able to streamline the diagnosis and management of this disease. “We are detecting *C. difficile* antigen and toxin in patients much quicker and earlier,” noted Prof. Delmée.

A major cause of hospital-acquired diarrhea is *C. difficile* in developed countries. Further, once there is a case of diarrhea in the hospital, the environment is rapidly contaminated by spores that may persist for weeks or longer, increasing the patient’s risk of exposure or re-exposure to *C. difficile*. Thus, management of *C. difficile* focuses on prompt and effective management of not only the patient but also the patient’s environment, both to improve individual patient outcomes and to prevent the spread of infection. To meet this need, an accurate and rapid diagnosis of every *C. difficile* infection is essential. According to Prof. Delmée, “Every case of
hospital-acquired diarrhea should be tested for C. difficile – it’s the main known cause.”

“Every case of hospital-acquired diarrhea should be tested for C. difficile – it’s the main known cause”

Screening of stool samples for C. difficile infection is recommended in all patients with at least one of the following:
- History of antibiotic therapy
- Hospital-acquired diarrhea
- Age >65 years
- History of C. difficile-associated diarrhea.

Careful consideration should be given to patients of advanced age, which is a risk factor for developing C. difficile-associated diarrhea.25 Residence in a nursing home represents the most important risk factor for developing diarrhea in older people.26 A recent survey of residents of a long-term care facility demonstrated under-diagnosis of C. difficile infection.27 By contrast, screening should be avoided in children aged less than 2 years and in patients with formed stools, as these patients are very unlikely to have C. difficile disease.

Available tests for C. difficile

For the past 30 years, the cytotoxicity assay and culture have been the reference tests for C. difficile infection. More recently, rapid tests, including toxin EIAs, GDH EIA, and PCR, have become available (Table 3).

Table 3 – Summary of available diagnostic tests for C. difficile infection

<table>
<thead>
<tr>
<th>Test</th>
<th>Time to detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference test</td>
<td></td>
</tr>
<tr>
<td>Cytotoxicity assay</td>
<td>6−48 h</td>
</tr>
<tr>
<td>Culture</td>
<td>24−48 h</td>
</tr>
<tr>
<td>Rapid test</td>
<td></td>
</tr>
<tr>
<td>Toxin EIA (A/A+B)</td>
<td>15−60 min</td>
</tr>
<tr>
<td>GDH EIA</td>
<td>15−60 min</td>
</tr>
<tr>
<td>PCR</td>
<td>2−3 h</td>
</tr>
<tr>
<td>EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; PCR, polymerase chain reaction</td>
<td></td>
</tr>
</tbody>
</table>

Cytotoxicity assay and culture

With the cytotoxicity assay, a sterile filtrate of a stool sample is added to a monolayer of tissue cultured cells and observed to identify the specific cytopathic effect of C. difficile infection. It appears often, within 6−48 hours. This is the most specific test for C. difficile infection, but it has only moderate sensitivity (60−70%) when compared to toxigenic culture. In addition, it is very time consuming, is technically difficult to accomplish, requires human cell cultures, and is not very well standardized.

Culture is still performed using the same cycloserine cefoxitin fructose agar media originally described in the 1970s.28 It is the most sensitive test available for diagnosing C. difficile infection and identifies virtually every positive case. Many laboratories no longer use this test owing to its labor-intensive and technical requirements, such as correct atmosphere, cell medium and pH. Once a colony is isolated, it can be tested for toxin production in about 20 minutes. This is termed “toxigenic culture”.

A prospective study evaluated the effectiveness of toxigenic culture in diagnosing C. difficile-associated diarrhea.29 Over 7 years, physicians at the Brussels Saint-Luc University Hospital collected 10,552 stool samples from 7,042 patients for analysis using toxigenic culture. A total of 1,058 samples (10%) tested positive in the culture, and 460 (43.4%) of these tested positive for fecal cytotoxin. The remaining 598 cultures were tested for toxigenicity, and 355 (59.4%) were found to be positive. Thus, toxigenic culture diagnosed over half of the cases of C. difficile-associated diarrhea that would have been missed using cytotoxicity assay alone. These results support a protocol using toxigenic culture in addition to routine cytotoxicity assay. They also demonstrate that toxigenic culture remains the “gold standard” in the evaluation of new diagnostic tests.

Rapid tests in the current clinical environment

In the 1990s, a series of EIAs for C. difficile were developed to improve accuracy and decrease turn-around time. Most of these assays detected Toxin A, but some were also designed to detect both Toxins A and B, as some strains producing only Toxin B have been reported in both Europe and North America.30,31 In addition, some EIAs were developed to detect GDH. Finally, PCR testing has also been used to detect C. difficile infection.

EIAs are easier to perform and much faster than traditional tests, yielding results in 20−40 minutes. Sensitivity with Toxin A and B EIAs is only slightly improved over cytotoxicity assays. GDH EIA has a much higher sensitivity (88.9%) compared with both EIAs and cytotoxicity assays. Specificity is low, however, yielding a low positive predictive value that makes the test unacceptable as a diagnostic test on its own (Table 4).32,33 Nevertheless, the negative predictive value is high, allowing the test to be used as a helpful screening tool. In 20 minutes, clinicians can exclude C. difficile infection as a possible diagnosis if the test is negative. By comparison, PCR testing yields both good sensitivity and specificity, but the test detects the toxin gene, rather than the toxin itself or living bacteria (Table 4). Further, PCR is expensive, limiting its usefulness in screening and diagnosis.

Dr Oppenheim and colleagues recently evaluated C. difficile detection by lateral flow assay for antigen and toxin (C. diff Quik Chek Complete®), an EIA (Toxin A&B assay alone), and two different commercial PCR methods. All four tests were performed on 150 consecutive liquid stool specimens collected from patients aged ⩾65 years who developed diarrhea at least 48 hours after hospital admission. Samples were also evaluated against two reference standards (cell cytotoxicity neutralization assay and toxigenic culture). Table 5 summarizes the performance of the four investigated tests compared with toxigenic culture.34

Table 4 – GDH versus PCR as C. difficile screening

<table>
<thead>
<tr>
<th>Test</th>
<th>GDH</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versus toxigenic culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>number of positive (+) fecal samples</td>
<td>GDH+ 16</td>
<td>21 PCR+ 44 7</td>
</tr>
<tr>
<td>number of negative (−) fecal samples</td>
<td>GDH− 2</td>
<td>161 PCR− 10 204</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>88.9</td>
<td>81.5</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>88.5</td>
<td>96.7</td>
</tr>
<tr>
<td>Positive predictive value, %</td>
<td>43.2</td>
<td>86.3</td>
</tr>
<tr>
<td>Negative predictive value, %</td>
<td>98.8</td>
<td>95.3</td>
</tr>
<tr>
<td>GDH, glutamate dehydrogenase; PCR, polymerase chain reaction; TC, toxigenic culture.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The C. diff Quik Chek Complete® two-step diagnostic algorithm

Similar to Prof. Wilkins, Prof. Delmé devised a simple algorithm for diagnosing C. difficile in the clinical setting. The algorithm was first designed as a three-step process, with GDH screening followed by Toxin A and B screening, and final diagnosis by toxigenic culture. Validation by toxigenic culture at each stage showed that, of the 295 total samples, three positive samples were missed by GDH screening and 10 positive samples were missed by Toxin A and B screening.

In a simplified, two-step protocol, patients are first screened for C. difficile infection with the combined GDH and Toxin A&B assay C. diff Quik Chek Complete®, followed by toxigenic culture or PCR testing in patients with contradictory results (Figure 3). Those with positive or negative results for both GDH and Toxins A and B are reported as positive or negative, respectively. This constitutes the vast majority of samples (80–90%), so that only a small proportion of samples must be further evaluated with additional testing. The protocol was validated with clinical testing and showed a sensitivity of 82.6%, a specificity of 98.1%, a positive predictive value of 82.6%, and a negative predictive value of 98.1%. A two-step algorithm for C. difficile detection using GDH and Toxin A&B assay followed by stool culture had a turnaround time of less than 4 hours for 92% of the specimens. A two-step algorithm involving GDH screening followed by PCR testing also showed a high degree of accuracy. Notably, only 20% of samples required PCR confirmatory testing.

Prof. Delmé concluded that C. diff Quik Chek Complete® is a rapid, cost-effective screening tool with an excellent negative predictive value, using combined GDH and Toxin A and B detection.

According to the 2010 practice guidelines for C. difficile infection in adults developed by the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America, a two-step method for diagnosis is recommended, in which EIA screening with GDH detection is followed by cytotoxicity assay or toxigenic culture as confirmation in positive samples.

<table>
<thead>
<tr>
<th>Table 5 – Performance of C. diff Quik Chek Complete®, Toxin A&amp;B assay alone, and PCR compared with toxigenic culture34</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>GDH</td>
</tr>
<tr>
<td>Sensitivity, %</td>
</tr>
<tr>
<td>Specificity, %</td>
</tr>
<tr>
<td>Positive predictive value, %</td>
</tr>
<tr>
<td>Negative predictive value, %</td>
</tr>
</tbody>
</table>

1 Two different commercial PCR tests (1 and 2) were evaluated.

CDT, C. difficile toxin; GDH, glutamate dehydrogenase; PCR, polymerase chain reaction.

“C. diff Quik Chek Complete® is a rapid, cost-effective screening tool with an excellent negative predictive value, using combined GDH and Toxin A and B detection”

Lactoferrin: biomarker for C. difficile and IBD

Prof. Jost Langhorst discussed new concepts in the role of lactoferrin in C. difficile infection and in IBD and the utility of lactoferrin level in assessing these patients.

C. difficile infection begins with alteration of normal gut flora due to nosocomial or community-acquired C. difficile followed by growth of the pathogen and production of toxins. Tissue damage and inflammation caused by Toxins A and B follow, resulting in diarrhea and colitis due to influx of neutrophils and fluids. Patients with IBD, including Crohn’s disease (CD) and UC, form a group who are especially vulnerable to gastrointestinal disease.

Identification of biomarkers helps in diagnosing patients with C. difficile-associated diarrhea and determining the severity of the inflammatory reaction. Lactoferrin is a glycoprotein found in many body fluids and is an important part of the secondary granules of activated neutrophils. During intestinal inflammation, activated neutrophils infiltrate the mucosa, increasing the level of lactoferrin in the intestinal lumen. Lactoferrin is a very useful biomarker, because it is stable in feces for several days at room temperature and for months at −20°C. As previously mentioned, research by Wren and colleagues demonstrated that lactoferrin level directly correlates with the severity of C. difficile infection. According to Prof. Langhorst, “As soon as inflammation caused by Toxins A and B appears and diarrhea and colitis take place, lactoferrin is measurably elevated in the feces.” According to Prof. Langhorst, lactoferrin level is a biomarker for intestinal inflammation, facilitating early intervention to ensure optimal outcomes.

Lactoferrin level is a biomarker for intestinal inflammation, facilitating early intervention to ensure optimal outcomes.
Lactoferrin and IBD

Ongoing research is investigating lactoferrin in IBD, particularly its role in monitoring treatment outcome and disease activity.39−45

The presence of lactoferrin differentiates active IBD from functional disease such as IBS. Prof. Langhorst and his colleagues showed an increased median lactoferrin level with inflammation in both active UC and CD patients compared to those with UC or CD in remission, or IBS and no inflammation (Table 6). The differences in lactoferrin level between UC or CD patients with active inflammation and patients with IBS were highly significant.46 “High lactoferrin significantly discriminates active inflammatory bowel disease from functional IBS,” noted Prof. Langhorst.

Vieira and colleagues showed that lactoferrin is an accurate marker of intestinal inflammation in both UC and CD, with an overall accuracy of 91% (sensitivity, 90%; specificity, 92%; positive predictive value, 96%; negative predictive value, 83%). These results were more accurate than those of the clinical symptom index and C-reactive protein (CRP), and similar to those of the CD Endoscopic Index of Severity for diagnosing intestinal inflammation.43

A separate study conducted by Prof. Langhorst and colleagues evaluated the combination of biomarker, symptoms, and serum markers in diagnosing UC and CD.44 Diagnostic accuracy of the combination of biomarkers such as lactoferrin, symptoms such as diarrhea or bloody stools, and CRP, was very high, especially for UC (95.3%). Diagnosis is more complicated in CD, which includes several subgroups of disease.

Lamb and colleagues monitored lactoferrin level postoperatively in patients with CD. In uncomplicated postoperative courses, lactoferrin level normalized within 2 months. In patients with active disease, the marker was elevated, and this proved to be more accurate in predicting clinical activity than were CRP, platelet count, or endoscopic appearance.42

Lactoferrin and C. difficile in IBD

The potential role of C. difficile as a confounding factor in the clinical course of IBD was first discussed by Trnka and colleagues three decades ago,46 and it remains a clinical dilemma today. Retrospective studies have shown an increase in the rate of C. difficile infection in patients with IBD, particularly those with UC; infection rates of IBD inpatients have doubled or even tripled in recent years.47−49 The link between C. difficile infection and IBD is inflammation (Figure 4). While lactoferrin level can indicate that inflammation is occurring, it cannot determine the cause.

Table 6 – Lactoferrin level in patients with UC, CD, and IBS46

<table>
<thead>
<tr>
<th></th>
<th>No inflammation</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>N</td>
<td>Median lactoferrin, μg/mL</td>
</tr>
<tr>
<td>No inflammation</td>
<td>15</td>
<td>4.34</td>
</tr>
<tr>
<td>Inflammation</td>
<td>27</td>
<td>51.1</td>
</tr>
<tr>
<td>CD</td>
<td>No inflammation</td>
<td>Inflammation</td>
</tr>
<tr>
<td>No inflammation</td>
<td>10</td>
<td>6.4</td>
</tr>
<tr>
<td>Inflammation</td>
<td>33</td>
<td>55.1</td>
</tr>
<tr>
<td>IBS</td>
<td>No inflammation</td>
<td></td>
</tr>
<tr>
<td>No inflammation</td>
<td>54</td>
<td>1.82</td>
</tr>
</tbody>
</table>

CD, Crohn’s disease; IBS, irritable bowel syndrome; UC, ulcerative colitis.

As with the general population, risk factors for C. difficile-associated diarrhea in patients with IBD include hospitalization and antibiotic therapy. In patients with IBD, immunomodulatory therapy represents an additional risk factor for C. difficile-associated diarrhea.49 The potential for IBD patients to harbor pathogenic bacteria may also serve as an independent predictor of C. difficile infection. C. difficile carriers (GDH-positive patients) may develop C. difficile infection over time. A prospective study showed C. difficile carrier status was more common in outpatients with IBD (8.2%), including UC (9.4%) and CD (6.9%), compared with outpatient controls (1%). Further, the higher carrier status rate remained, regardless of known risk factors for C. difficile acquisition.51

Prof. Langhorst and colleagues conducted an additional study, which was presented at the 18th UEGW.52 Among 92 patients with UC in clinical remission not receiving immunosuppressive medication, 7% were C. difficile carriers (GDH positive). Among these, 66% exhibited elevated lactoferrin levels, indicating at least low-grade inflammation. Of the entire study population of 92 patients, 45 (49%) experienced a flare-up within 12 months. Of these patients, 11% were C. difficile carriers (GDH positive), and all showed elevated lactoferrin levels, consistent with a flare-up. Of note, none of the C. difficile carriers were positive for C. difficile infection by cytotoxicity assay, and none of the carriers either in remission or during a flare-up required additional treatment with antibiotics. Thus, the presence of C. difficile without stool toxin was a secondary phenomenon, rather than a risk factor for relapse, in UC outpatients not receiving immunosuppressive medication. None of these patients experienced C. difficile infection, demonstrating the potential for over-treatment of C. difficile with antibiotics.

Summary and conclusions

Interest in the management of C. difficile infection remains high as rates of infection increase in both Europe and North America, and as new detection methods become available. Cytotoxicity assay and toxigenic culture remain the reference tests for diagnosis, but these methods require up to 48 hours for completion. Rapid screening tests include toxin ELISA and GDH EIA, which can be completed in less than 1 hour, and PCR, which can be completed in less than 3 hours. While PCR yields both good sensitivity and specificity, the test is expensive to perform. In a simple, two-step algorithm, combined GDH and Toxin A&B
screening is followed by toxigenic culture or PCR only in samples that are only positive for GDH in the initial screening.

Lactoferrin is an important biomarker in *Clostridium difficile* infection. Lactoferrin level increases with neutrophil infiltration in the intestines and is proportional to the degree of intestinal inflammation. The protein is a valuable biomarker of inflammation in *Clostridium difficile*-associated diarrhea, and may be useful in combination with *C. difficile* Quik Chek Complete® GDH/Toxin A&B screening to diagnose infection.

Lactoferrin level can differentiate inflammatory from functional gastrointestinal disorders, and may detect and quantify intestinal inflammation in IBD. Recent results show that *Clostridium difficile* carrier status is more common in inpatients as well as outpatients with IBD, and that this may be a secondary phenomenon, rather than a risk factor for *Clostridium difficile* infection, in patients not receiving immunosuppressive medication. Thus, in patients with active IBD, diagnosis of *Clostridium difficile* requires proof of stool toxin, even with elevated levels of lactoferrin.

### References


Techlab C. DIFF QUIK CHEK COMPLETE® is the only device available to simultaneously detect Clostridium difficile glutamate dehydrogenase (GDH) antigen and toxins A & B in a single reaction. It is an accurate and easy to use method with results within 25 minutes and an NPV of 99%.

When Techlab C. DIFF QUIK CHEK COMPLETE® is used with a Techlab Lactoferrin test such as LEUKO EZ VUE, it is a cost effective way of giving patients a faster diagnosis, while reducing the need for PCR.

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