Importance of Stool Toxin Testing: Are We Coming Full Circle?

In recent years, diagnostic testing for *C. difficile* disease has evolved significantly to include new approaches like the use of molecular assays for detecting the toxin genes (*tcdA* or *tcdB*) and the inclusion of glutamate dehydrogenase (GDH)-based algorithm testing for identifying *C. difficile* negative specimens. As with any diagnostic testing, there are pros and cons associated with both approaches. The molecular tests are highly sensitive and offer an assay for determining the presence of toxigenic *C. difficile* in a fecal specimen. For larger institutions that can absorb the increased cost, molecular testing for all incoming specimens is sometimes used as a single test approach. Toxigenic culture, which is the gold standard for identifying the presence of toxigenic *C. difficile,* involves subculturing isolates in broth media like brain heart infusion broth (BHI), and then confirming the production of toxin B with tissue culture or by immunoassay. This procedure takes days to complete and requires additional laboratory capabilities that are beyond many clinical labs. The molecular assays provide a result the same day and some studies show a >90% correlation with toxigenic culture.

Algorithm testing uses GDH for determining the presence of *C. difficile,* followed by additional testing using stool toxin or molecular assays to differentiate between toxigenic and nontoxigenic infections. Since most specimens are negative (ca. 80%), this testing approach significantly decreases the high cost of the molecular tests. Algorithm testing can be done using several combinations. First, specimens may be screened for GDH using a standalone ELISA test like the *C. DIFF CHEK-60*™ followed by positive specimens requiring testing using a molecular assay for the toxin genes or immunoassay for stool toxin.

Another approach is using the *C. DIFF QUIK CHEK COMPLETE*® test for determining the presence of both GDH and stool toxin with a single test. With this test, specimens that are GDH and stool toxin-positive can be reported as “positive” and then GDH-positive toxin-negative specimens can be further tested by a molecular test for the presence of toxigenic *C. difficile*. This approach is rapid, eliminates the negative specimens, provides a stool toxin result, and ultimately lowers costs.

With the different options for testing, it’s important to consider what these different tests mean and how they impact patient care. A recent study presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) by Planche *et al.* entitled “Clinical Validation of *Clostridium difficile* Infection (CDI) Diagnostics: Importance of Toxin Detection” presented results from the largest *C. difficile* study to date. In this U.K. study,

12,420 specimens from 10,691 patients were tested using the reference assays: toxigenic culture and tissue culture for stool toxin along with ELISA tests for GDH and toxin and a molecular test. Of the total patients, there were 6,524 inpatients of which 5,927 survived. According to the reference assays, there were 3 groups described: (1) toxigenic culture and tissue culture stool toxin-positive, (2) toxigenic only positive, and (3) *C. difficile* negative. Diagnostic status was evaluated for an association with mortality rate, and a multivariate analysis between groups was done with age, sex, clinic location, white blood cell count, creatinine and albumin as covariates.

There were 435 stool toxin-positive patients (7%), 207 patients positive by only toxigenic culture (3%) and 5,880 patients who were negative for *C. difficile* (90%). Patients with stool toxin had a significantly higher mortality rate (16.6%) compared to the toxigenic only (9.7%; $p<0.022$) and *C. difficile* negative groups (8.6%; $p<0.001$). In addition, the stool toxin-positive patients had significantly higher ($p<0.001$) WBC counts compared to the other two groups. Based on these results, Planche and co-authors concluded that patients having a positive stool toxin result have *C. difficile* disease with an increased risk of mortality. In addition, they defined a new diagnostic category: patients who are infected with toxigenic *C. difficile* without stool toxin are “excretors” for whom *C. difficile* disease is unlikely but a risk of transmission should be considered. Since overtreatment may result in additional antibiotic resistance along with potentially putting patients at risk of developing the disease rather than just carrying the organism, many hospitals will take note of this study and again ask the question: Should stool toxin testing be included to optimize their *C. difficile* diagnostic testing? With the continued evolution of *C. difficile* disease, determining the best approach to both diagnosis and treatment continues to be an ongoing dilemma.

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Literature Cited

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