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1 Point-Counterpoint: What is the optimal approach for detection of *Clostridium difficile*

2 infection?

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Running title: Optimal approach for CDI detection

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30 In 2010, we published an initial point-counterpoint on laboratory diagnosis of *C. difficile*
31 infection (CDI). At that time, nucleic acid amplification tests (NAATs) were just becoming
32 commercially available, and the idea of algorithmic approaches to CDI was being explored.
33 Now there are numerous NAATs in the marketplace and based on recent proficiency test
34 surveys, they have become the predominant method used for CDI diagnosis in the United States.
35 At the same time, there is a body of literature that suggests that NAATs lack clinical specificity
36 and thus inflate CDI rates. Hospital administrators are taking note of institutional CDI rates
37 because they are publicly reported. They have become an important metric impacting hospital
38 safety ratings and value-based purchasing where hospitals may have millions of dollar of
39 reimbursement at risk. In this point-counterpoint using a Frequently Asked Question approach,
40 Ferric Fang of the University of Washington, who has been a consistent advocate for NAAT-
41 only approach for CDI diagnosis, will discuss the value of a NAAT-only approach, while
42 Christopher Polage of the University of California-Davis and Mark Wilcox of Leeds University,
43 UK, who have each recently written important articles on the value of toxin detection in the
44 diagnosis, will discuss the impact of toxin detection in CDI diagnosis.

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46

47 **Frequently Asked Questions**

48 **1. Why is there so much controversy about the performance of *C. difficile* diagnostic tests?**

49 **Fang-** Diagnostic tests detect either toxigenic *C. difficile* or its toxins. Many labs have switched
50 from toxin assays to NAATs that detect toxigenic *C. difficile* in order to maximize
51 sensitivity, as toxin assays were previously missing cases of clinically significant CDI.
52 However some recent studies have highlighted that NAATs can be positive in colonized
53 patients without disease, and patients with positive toxin assays may have a worse prognosis
54 than those with a positive NAAT only (1, 2). This has renewed controversy about the
55 optimal approach to diagnosis CDI.

56 **Polage and Wilcox-** The performance of *C. difficile* diagnostic tests is controversial for 4
57 reasons:

- 58 1) There is no reliable clinical or laboratory definition for CDI that accurately distinguishes true
59 CDI from non-CDI-related symptoms in all patients (3). Most diarrhea in hospitals is not due
60 to CDI and virtually all clinical signs and symptoms of CDI are non-specific and occur
61 commonly in patients without CDI (4, 5). Asymptomatic *C. difficile* colonization is also
62 common in hospitals, particularly among patients who get selected for *C. difficile* testing due
63 to shared risk factors between colonization and CDI (6, 7). Thus, the positive predictive
64 value of detecting toxigenic *C. difficile* in routine diarrheal samples submitted to the
65 laboratory is low and insufficient to diagnose CDI (1-3, 7).
- 66 2) The measured performance of *C. difficile* diagnostic tests is highly dependent on the
67 definition of CDI and ratio of CDI to colonization in the population being tested (2, 3, 8). For
68 example, toxin tests are sensitive (and agreement with toxigenic culture is high) in patients
69 with pseudomembranous colitis due to the high ratio of CDI to colonization in this

70 population (8). Conversely, toxin tests appear less sensitive in routine stool samples
71 submitted to the laboratory due to frequent overlap of non-CDI diarrhea with *C. difficile*
72 colonization and the lower ratio of CDI to colonization in this population (1-3, 8,9).

73 3) Anecdotal experiences with cases of severe CDI missed by toxin tests have promoted a desire
74 for absolute sensitivity regardless of specificity and an erroneous belief that all patients with
75 toxigenic *C. difficile* and diarrhea have CDI as the cause of their symptoms (9-14).

76 Widespread misclassification of non-CDI diarrhea in patients with *C. difficile* colonization as
77 ‘CDI’ has reinforced the belief that toxin tests are insensitive for CDI without systematic
78 investigation to verify the true frequency of disease (2, 9, 11, 15-17).

79 4) *C. difficile* tests vary in performance accuracy, including those with the same target; for
80 example, there are marked and sometimes significant differences in sensitivity and specificity
81 between commercial toxin detection tests (1, 3, 9). Thus, use of less well performing tests
82 can reinforce perceptions driven by other factors (above).

83 **Editor’s comment:** The measured accuracy of any diagnostic test is dependent upon the
84 reference test to which the diagnostic test is being compared. The American Society for
85 Microbiology has a group that is currently working on an evidence based practice guideline for
86 laboratory detection of *C. difficile* infection. There are over 15 different reference methods that
87 have appeared in this literature some of which are clearly biased. This lack of a standard
88 reference method to define *C. difficile* infection clearly complicates an already very complicated
89 literature and there is no consensus in sight.

90

91 **2. What are the effects of using nucleic acid amplification testing for *C. difficile* on *C.***
92 ***difficile* infection data that institutions report to public health authorities?**

93 **Fang-** Since NAATs are more sensitive than toxin assays, the introduction of a NAAT will
94 initially increase the apparent infection rate at an institution. However, this is mitigated by
95 two factors. First, the National Health Safety Network applies a correction factor for
96 institutions that use NAATs to diagnosis CDI, so that institutions using more sensitive
97 diagnostic methods will not be penalized (18). Second, the greater detection of toxigenic *C.*
98 *difficile* by NAATs can facilitate more effective infection control measures so that
99 institutional infection rates subsequently decline (19-21). This has been the experience at my
100 own institution, where several years ago our CDI rates fell within a few months of
101 introducing NAAT and have remained low ever since. The sensitive detection of toxigenic
102 *C. difficile* can facilitate efforts to reduce institutional transmission. That said, public health
103 agencies must recognize that laboratory data alone cannot be used to accurately monitor CDI
104 rates, as laboratory tests detect both colonized and infected patients.

105 **Polage and Wilcox-** When positive laboratory test results are used as the sole measure of
106 healthcare facility-onset CDI – as is currently the case for most hospitals in the United States
107 – NAAT-based CDI diagnosis can have a dramatic effect on the number of CDI cases
108 institutions report publically and affect hospital reimbursement under value-based payment
109 programs (18, 22-24). This is because NAAT-based CDI testing results in public reporting of
110 all fecal toxin-negative samples with toxigenic *C. difficile* as positive regardless of clinical
111 disease or treatment. Most hospitals using NAAT or GDH immunoassay plus NAAT for CDI
112 diagnosis see an increase in the number of ‘CDI cases’ reported publically by 1.5 to 3-fold
113 over rates derived from toxin tests (18, 23, 24). The NAAT-related increase is partially
114 accounted for by an adjustment in the NHSN standardized infection ratio (SIR) calculation
115 used to compare hospital performance, but the current adjustment does not fully correct for

116 the increased number of positive results at all hospitals (24). This might be appropriate if all
117 toxin-negative patients with *C. difficile* detected by NAAT had CDI clinically, but this is not
118 the case (2, 3, 8). Recent outcome studies show that most toxin-negative patients with *C.*
119 *difficile* detected by NAAT or culture recover spontaneously without treatment and have a
120 significantly lower rate of adverse events than toxin-positive patients; furthermore, the
121 duration of symptoms for toxin-negative patients with *C. difficile* detected by NAAT is
122 similar to that for *C. difficile*-negative control patients (1, 2, 25). These findings suggest that
123 using NAAT as a standalone test for CDI diagnosis results in a considerable amount of over-
124 diagnosis that has important clinical, financial, and reputational implications for hospitals (2,
125 25). For this reason, guidelines in the UK and Europe now recommend toxin testing to
126 confirm CDI in NAAT-positive patients, and consideration of other causes for symptoms
127 before diagnosis and treatment of CDI in toxin-negative patients (3).

128

129 **3. Should GDH immunoassays be used as a screening test to determine which stool**
130 **specimens should be subjected to toxin or nucleic acid amplification testing for *C.***
131 ***difficile*?**

132 **Fang-** GDH immunoassays are more sensitive than toxin assays and can be used to screen
133 specimens for the presence of *C. difficile* (26). However GDH is expressed by both toxigenic
134 and non-toxigenic strains of *C. difficile*, so GDH-positive specimens must be further tested
135 using NAAT and/or toxin assays. Such an approach is less expensive than performing
136 NAAT on all specimens but is also less sensitive, particularly for non-027 strains (27, 28).
137 This is not because of strain-dependent differences in GDH expression but most likely

138 because 027 strains tend to attain higher organism burdens. The calculated sensitivity of the
139 GDH immunoassay is dependent on the sensitivity of the comparator method, and studies
140 including a blinded multi-center trial using the most sensitive comparators (NAAT and
141 toxigenic culture with detection of both spores and vegetative cells) have shown that GDH
142 assays miss approximately 20% of specimens detected by NAAT in patients with
143 symptomatic CDI (17, 27, 28). In short, a GDH-based algorithm is less costly but sacrifices
144 sensitivity.

145 **Polage and Wilcox-** GDH detection is sensitive for CDI because *C. difficile* vegetative cells
146 express and secrete GDH extracellularly, and GDH may play a role in *C. difficile*
147 colonization *in vivo* (29). As a result, most clinical samples with toxigenic *C. difficile*
148 detectable by culture or NAAT are positive by GDH immunoassays and virtually all samples
149 with toxins detectable are positive for GDH (3, 9, 30). The occasional samples that are
150 positive by NAAT but negative for GDH have a low concentration of *C. difficile* and no
151 toxins, suggesting that these are most likely *C. difficile* carriers or patients on treatment (30).
152 Most laboratory comparisons find that GDH immunoassays are >90% sensitive for *C.*
153 *difficile*, as confirmed by two meta-analyses; a few studies report slightly lower sensitivities
154 in the range of 83.1-87.6% (3, 9, 26). In the most recent meta-analysis, the pooled sensitivity
155 of GDH immunoassays was 94% (95% CI, 89-97%) and 96% (95% CI, 86-99%) relative to
156 cell cytotoxin neutralization assay and toxigenic culture, respectively; the pooled specificity
157 was 90-96% (3). Finally, recent studies showed that GDH expression is a reliable
158 characteristic of all common *C. difficile* strains, contradicting an earlier study, which
159 hypothesized that differential GDH expression might explain the lower sensitivity of two-
160 step immunoassay algorithms for some *C. difficile* ribotypes (9, 27). In summary, GDH

161 immunoassays are less expensive and modestly less sensitive as a screening test than some
162 NAAT; NAAT are generally more sensitive, specific, and expensive. Samples that test
163 positive by either method should be retested by a fecal toxin A/B immunoassay to confirm
164 clinical CDI disease (3). Individual laboratories should choose the *C. difficile* screening test
165 and algorithm that works best in their lab and institution.

166

167 **4. What is the most cost-effective strategy for *C. difficile* diagnosis?**

168 **Fang-** Although immunoassay methods are less costly for the laboratory than NAATs, a recent
169 cost-effectiveness analysis has determined that NAAT is the most cost-effective approach
170 from an institutional standpoint due to the \$9,000 to \$13,000 cost of each missed case of CDI
171 (31). Another study found that patients diagnosed with CDI by NAAT had a two-day shorter
172 median length of stay compared to patients diagnosed by toxin immunoassay, even though
173 the patients did not differ with regard to co-morbidity, prior hospitalizations, laboratory
174 parameters or mortality (32). Length of stay is an important contributor to the financial costs
175 of CDI (33, 34), and the authors suggested that the sensitive NAAT assay might result in
176 more timely diagnosis and treatment (32). In addition, reliance on a less sensitive diagnostic
177 method may lead to more empiric therapy (35) and repeat laboratory testing, because
178 clinicians lack confidence in a negative result. Thus, the use of NAAT can promote
179 responsible antimicrobial stewardship and reduce unnecessary antibiotic and laboratory
180 utilization.

181 **Polage and Wilcox-** The latest guidelines recommend a two or three-step algorithm as the most
182 effective strategy to diagnose CDI and minimize over-diagnosis of *C. difficile* colonized
183 individuals who have other causes of their diarrheal symptoms (3). The algorithm should

184 start with a rapid and sensitive screening test with high negative predictive value for CDI,
185 such as a GDH immunoassay or NAAT, to minimize empiric isolation and treatment of non-
186 CDI patients (3). Samples with a positive screening test should be retested with a toxin A/B
187 immunoassay to identify patients with toxins, who have the highest likelihood of CDI
188 clinically and need for treatment (3). Patients with toxigenic *C. difficile* but no fecal toxins
189 need additional clinical evaluation to distinguish incidental *C. difficile* colonization (most
190 patients) from CDI with a negative toxin test (fewer patients) (3). The overall sensitivity and
191 specificity of this approach was verified in a multicenter prospective study in the UK and
192 supported in a recent meta-analysis (1, 3). The emphasis on fecal toxin detection in this
193 algorithm to identify patients with high (toxin-positive patients) and low (toxin-negative
194 patients) likelihoods of clinical CDI disease is supported by outcome studies in multiple
195 countries (1, 2, 8, 25). In terms of cost, new economic models are needed to determine which
196 strategy is best since previous models inappropriately assumed that patients with toxigenic *C.*
197 *difficile* and negative toxin tests had CDI and overlooked the costs of CDI over-diagnosis,
198 including decreased hospital reimbursement (31, 36).

199 **Editor's comment:** A March 2016 survey of 70 members of Clinmicronet, a global list serve of
200 doctoral clinical microbiologists showed that 55 laboratories used a NAAT only approach
201 while 9 used a GDH/toxin screen with PCR confirmation for GDH/toxin discrepant
202 specimens. CAP surveys of *C. difficile* testing also show a preponderance of laboratories
203 using a NAAT only approach. Only 6 of 70 respondents used the algorithm of a GDH or
204 NAAT screen with toxin confirmation of screen positive results described by Polage and
205 Wilcox. Three laboratories were considering changing to this approach. One microbiologist

206 commented that the decision to change to this algorithm was driven by hospital
207 administration belief that using this approach would reduce reported CDI rates.

208

209 **5. Why do studies of symptoms and clinical outcomes in patients who have *C.***
210 ***difficile* DNA or bacteria but not toxins in stool reach such different conclusions?**

211 **Fang-** NAATs and culture-based methods are more sensitive but less specific, whereas toxin
212 assays are less sensitive but more specific. Thus, patient selection is critically important for
213 the proper interpretation of test results. With regard to specificity, it is important to
214 recognize that no *C. difficile* diagnostic assay is completely specific for clinical disease.
215 Production of toxin is essential but not sufficient for disease, and even patients with high
216 fecal toxin levels may be asymptomatic (37, 38), particularly if they have toxin-neutralizing
217 antibodies (39). With regard to sensitivity, it is equally important to recognize that toxin
218 assays can be negative in patients with symptomatic (and even life-threatening) CDI (10, 13,
219 40, 41). The insensitivity of toxin assays has been demonstrated even for cases of
220 pseudomembranous colitis and was a major consideration leading to the development of
221 more sensitive NAAT assays. In fact, a false-negative toxin assay is a risk factor for a fatal
222 outcome in patients with fulminant CDI (10), and I note that one of the toxin-negative
223 patients in the Polage study (2) "had recurrent CDI as a contributing factor to death." The
224 bottom line is that a negative toxin assay cannot rule-out the possibility of CDI. On the other
225 hand, the greater sensitivity of NAAT or culture-based diagnostic methods can increase the
226 likelihood of false-positive results, particularly in patients with a low clinical probability of
227 *C. difficile*-associated disease. Exclusion of patients who fail to meet the clinical definition

228 of diarrhea (or have formed stools), are receiving laxatives, or have previously tested
229 positive, can help to reduce the number of false-positive results. The best way to avoid false-
230 positive test results is to restrict diagnostic testing to patients who have clinical presentations
231 consistent with CDI, and inappropriate testing can account for many of the reported instances
232 of "overdiagnosis" (1, 2). Institutional guidelines with clear criteria for diagnostic testing can
233 be helpful in this regard.

234 Some have advocated the performance of both NAAT and toxin assays to optimize
235 patient management. However the data are conflicting. Although some studies suggest that
236 patients with positive toxin assays have a worse prognosis than those with positive NAAT
237 only (1, 2), many other carefully conducted studies involving more than 2,000 patients have
238 not found toxin assays to be predictive of symptoms, disease severity, mortality,
239 transmissibility or recurrence (15, 16, 38, 42-44). In any case, whether the detection of toxin
240 is indicative of a worse prognosis is beside the point. The notion that a toxin assay can
241 distinguish between colonization and infection is fundamentally flawed-- the distinction
242 between colonization and infection is a clinical one and cannot be based on laboratory
243 assessment alone. As Dubberke and Burnham have noted, one must "treat the patient, not the
244 test" (45). Some patients with positive toxin assays have asymptomatic colonization (37,
245 38), and some patients with negative toxin assays have CDI (10, 13, 15, 16, 40-44). More
246 than half of patients with symptomatic CDI would be missed by reliance on a toxin
247 immunoassay (15, 16, 42-44), an unacceptably high proportion of false-negative results.
248 Furthermore, patients with NAAT-positive/toxin-negative specimens may convert to toxin-
249 positive on re-testing; this was observed in 21% of individuals undergoing re-testing in the
250 Polage study (2). I recommend using a negative NAAT to rule-out the possibility of CDI and

251 a positive NAAT to indicate the possibility of CDI in a patient with a compatible clinical
252 presentation; using this approach, toxin assays are unnecessary. Treatment decisions should
253 be based on clinical assessment and the presence or absence of toxigenic *C. difficile*, not on
254 the ability or failure to detect fecal toxin.

255 I feel compelled to point out a self-contradiction in the European guidelines that advocate
256 toxin testing. On one hand the guidelines acknowledge that "the decision to treat CDI is
257 ultimately a clinical decision. . . treatment should not be withheld on the basis of laboratory
258 tests alone"-- but on the other hand, they state that "using NAAT as a stand-alone test and
259 relying on clinical symptoms to discern patients from CDI from asymptomatic carriers is not
260 an optimal approach. . . samples with a positive result should be tested further with a toxin
261 EIA" (3). On what should treatment decisions be based, clinical assessment or the presence
262 of toxin? No wonder clinicians are confused.

263 I strongly disagree with the suggestion that a negative toxin assay means that a patient is
264 only colonized and not infected (1); such a simplistic approach is likely to result in the under-
265 diagnosis of CDI and harm to patients. Although some suggest that symptomatic patients
266 with CDI and negative toxin assays have self-limited disease that will resolve without
267 treatment (1, 2), this cannot be concluded from the available studies, as many of the patients
268 in these studies who had negative toxin assays received empiric treatment for CDI.
269 Furthermore, important clinical endpoints other than mortality, such as the duration and
270 severity of symptoms, were not measured, and the length-of-stay for culture-positive/toxin-
271 negative patients was actually significantly longer compared to controls with both tests
272 negative (1). Quite simply, the safety of withholding antimicrobial treatment from
273 symptomatic patients with positive NAAT and negative toxin assay results has not been

274 established. Untreated patients will also continue to shed *C. difficile* with the potential to
275 transmit infection to others, in contrast to those receiving specific antimicrobial treatment
276 (46).

277 **Polage and Wilcox-** There is a growing consensus that most patients with *C. difficile* DNA or
278 bacteria but no fecal toxins (i.e., toxin-negative/*C. difficile*-positive) are clinically distinct
279 from toxin-positive patients, have better outcomes, and generally do not have CDI as a cause
280 of their symptoms (1-3, 25). Overall, 14 of 18 studies (78%) have reported a clinical
281 symptom or outcome difference in toxin-negative/*C. difficile*-positive patients and large
282 studies from multiple countries have found less severe disease, a shorter duration of diarrhea,
283 fewer CDI-related complications, and/or lower mortality in these patients (1, 2, 8, 11, 15-17,
284 25, 43, 44, 47-54). In several studies, outcomes were similar to negative controls despite
285 delayed or non-reporting of NAAT or culture results and delayed or no treatment for CDI,
286 further supporting an alternate cause of symptoms (not CDI) (1, 2, 8, 47, 53).

287 Nonetheless, some studies reach the opposite conclusion - that toxin-negative/*C. difficile*-
288 positive patients have CDI and are not different from toxin-positive patients - and it is
289 important to understand how and why this might occur (11, 15-17, 43, 49). Most of these
290 studies were not adequately designed or powered to detect a statistical difference in rare
291 clinical outcomes, such as CDI-related complications or mortality and erroneously interpret a
292 non-significant *P*-value as evidence that differences do not exist (a type II statistical error)
293 (11, 15-17, 49). Many of these studies also have significant sources of bias, which likely
294 contributed to the authors' conclusions, including clinical reporting or reviewer knowledge of
295 NAAT results, and automatic classification of patients with positive NAAT or culture as
296 having CDI regardless of disease status (11, 15-17, 43, 49). Another common problem is

297 failure to acknowledge that many clinical signs and outcomes seen in patients tested for CDI
298 are common and non-specific in hospitals, and so are not necessarily indicative of, or related
299 to CDI (e.g., diarrhea, leukocytosis, ICU care) (11, 16, 49). Pre-analytic issues can also cause
300 negative results. One study routinely placed fecal samples in Cary-Blair transport media
301 before toxin testing, making it likely that pre-analytic dilution contributed to negative toxin
302 EIA results and so masked the relationship between fecal toxins and CDI-related outcomes
303 (43). In summary, there are good explanations for why some studies fail to find differences
304 between toxin-positive and toxin-negative/*C. difficile*-positive patients, and understanding
305 how and why such misinterpretations occur is critical to interpreting the literature in this
306 controversial field.

307 **Editor's comment:** Because of the uncertainty of which testing approach is most accurate in
308 predicting that a patient has CDI, it is clear that pre-analytic considerations are essential in
309 determining who should be tested for CDI. Ensuring that tested patients have documented
310 diarrheal disease and have not received laxatives in the past 48 hours is essential for
311 diagnostic accuracy regardless of testing approach.

312

313 **6. Will increasing the sensitivity of assays for *C. difficile* toxins in stool increase the**
314 **accuracy of toxin assays?**

315 **Fang-** Not necessarily. Toxin assays with increased sensitivity may reduce the incidence of
316 false-negative results. However, *C. difficile* toxins are labile at body temperature and
317 susceptible to inactivation by digestive enzymes (55, 56), so a completely sensitive toxin-
318 based assay may not be feasible. Even recent "ultra-sensitive" toxin assays are still less
319 sensitive than NAATs (57). The likelihood of clinical illness in individuals with positive

320 NAAT and negative ultra-sensitive toxin assay results remains to be determined. It should
321 also be noted that improvements in the sensitivity of toxin assays will not solve the issue of
322 false-positive results (i.e., specificity), which can be seen with any *C. difficile* diagnostic
323 method.

324 **Polage and Wilcox-** Maybe. Higher sensitivity toxin assays will decrease the number of CDI
325 cases 'missed' by toxin tests and bring the analytical and clinical performance closer to the
326 traditional cell cytotoxin neutralization assay (2, 30, 57, 58). This should be a good thing.
327 However, lowering the threshold for positive results will also decrease the specificity for CDI
328 and lead to classifying patients with transient or low levels of toxin due to *C. difficile*
329 colonization and antibiotic exposure as (likely erroneously) having disease (2, 57, 58). It is
330 not known whether detecting and treating these additional patients 'labelled' as having CDI
331 is necessary or beneficial (or possibly harmful) since most resolve their symptoms with
332 minimal or no treatment (2). These issues could be addressed by quantifying the level of
333 toxins to help physicians determine the likelihood that each patient has disease and warrants
334 treatment (57, 58). In any case, the overall diagnostic accuracy will depend on the test
335 performance characteristics *in the population being tested*. Test performance and diagnostic
336 accuracy are affected by many factors including local testing practices, use of diarrheagenic
337 medications, and the prevalence of CDI, *C. difficile* carriage, non-CDI diarrhea, anti-toxin
338 antibodies, and individual *C. difficile* strains in the population (5, 7, 59). Thus, high-
339 sensitivity toxin tests will probably improve diagnostic accuracy in hospitals/units with good
340 *C. difficile* testing practices, a low prevalence of *C. difficile* carriage, and low prevalence of
341 non-CDI diarrhea. However, diagnostic accuracy could easily be worse in hospitals/units
342 with indiscriminant *C. difficile* testing and a high prevalence of *C. difficile* carriage and non-

343 CDI diarrhea. Overall, accurate diagnosis of CDI depends on a multitude of factors and starts
344 at the bedside with good clinical evaluation of the likelihood of CDI and non-CDI diarrhea
345 and appropriate sampling and testing. Having a high sensitivity toxin test will definitely be
346 an improvement, but will not remove the need for laboratories to work with clinicians and
347 nurses to optimize clinical evaluation, testing, and diagnosis of symptomatic patients.
348

349 **7. Should the diagnostic testing strategy for *C. difficile* infection be different in oncology,**
350 **transplant and other immunocompromised patients?**

351 **Fang-** Immunocompromised hosts are at increased risk for CDI, and at least some studies
352 suggest comparable clinical severity of CDI in immunocompromised patients with positive
353 toxin assays and those with positive NAAT only (15, 49). However, as I advocate the use of
354 NAAT to diagnosis CDI in all patients, immunocompromised patients do not require a
355 special testing approach.

356 **Polage and Wilcox-** No. The two-step algorithm recommended in European guidelines is still
357 preferred in oncology, transplant and immunocompromised patients (3). Moreover,
358 diagnostic strategies based solely on detection of toxigenic *C. difficile* (e.g., NAAT only) are
359 likely to perform worse in these patients due to high rates of treatment-related diarrhea and
360 *C. difficile* carriage (5, 60). The lower positive predictive value of detecting toxigenic *C.*
361 *difficile* when diarrheal symptoms occur in these patients reinforces the need for judicious
362 testing, thoughtful clinical evaluation, and fecal toxin testing to maximize the accuracy of
363 CDI diagnoses in these groups (3, 5, 60).

364

365 **8. What is the significance of asymptomatic carriage of toxigenic *C. difficile*?**

366 **Fang-** Asymptomatic colonized patients are an important source of *C. difficile* transmission (6,
367 61) and are at substantially increased personal risk for the eventual development of
368 symptomatic CDI (62, 63). Therefore the identification of asymptomatic carriers can
369 enhance infection control and prevention efforts. A recent study suggests that detection and
370 isolation of colonized patients can prevent hospital-acquired CDI (64), and a CDC analysis
371 has concluded that reduced transmission due to the isolation of carriers was responsible for
372 the reduction in CDI incidence (65). High-risk antibiotics (e.g., cephalosporins,
373 fluoroquinolones, clindamycin) should be avoided if at all possible in patients known to carry
374 toxigenic *C. difficile*, and the possibility of CDI should be immediately considered if
375 diarrhea, fever or other compatible symptoms develop.

376 **Polage and Wilcox-** Asymptomatic *C. difficile* carriers outnumber CDI patients by at least 5 to 1
377 in most hospitals and are likely to be an important source of nosocomial *C. difficile*
378 transmission and infection (6, 7, 62, 64). A few studies have linked asymptomatic carriers to
379 a third or more of hospital-onset CDI cases (6, 7, 61). These observations have sparked an
380 interest in screening and isolation of asymptomatic carriers as a strategy to decrease
381 healthcare-associated CDI (6, 7, 64). So far, a single before-and-after study has been
382 published with results suggesting that screening may be effective (64). However, the current
383 absence of proven interventions for asymptomatic colonization and potential ramifications of
384 isolating large numbers of patients emphasizes the need for larger, well-controlled, multi-
385 center studies to confirm the effectiveness of screening before widespread adoption (7, 64).

386 Asymptomatic *C. difficile* colonization may also be an important predisposing risk factor
387 for CDI, but the story is somewhat mixed (59, 62, 66). Studies from the 1990s associated
388 lack of symptoms after *C. difficile* acquisition with pre-existing anti-toxin antibodies and

389 prior asymptomatic *C. difficile* colonization with lower risk of CDI in hospitals (59, 66).
390 These studies promoted the belief that most asymptomatic *C. difficile* carriers were immune
391 to *C. difficile* toxins but the high rate of colonization with a non-toxigenic *C. difficile* strain
392 (which also protects against CDI) was a potential confounder in one often mentioned review
393 (59, 66). More recently, asymptomatic *C. difficile* colonization has been associated with an
394 increased risk of CDI, but it is unclear if this is an artifact of NAAT testing, a change in the
395 epidemiology and pathophysiology of CDI, or simply a reflection of differential risk
396 according to the toxigenic status of colonizing strains (62). Hence, more work is needed to
397 determine the relationship between asymptomatic *C. difficile* carriage and subsequent risk of
398 CDI.

399 Finally, as noted above, asymptomatic *C. difficile* colonization is probably an important
400 source of erroneous CDI diagnoses in hospitals using *C. difficile* tests with poor predictive
401 value for CDI, as colonized patients with diarrheal symptoms due to medications, underlying
402 disease, and other infectious agents will yield positive (misleading) results (2, 5, 7, 67-69).

403

404 **Editor's comment:** One of the ongoing discussions concerning *C. difficile* is if admission
405 screening has any benefit. If asymptomatic patients are found to be colonized, they would
406 likely to be isolated since there are data suggesting colonized patients may spread *C. difficile*.
407 Although limiting the use of "high risk" antimicrobials in colonized patients is an attractive
408 idea, whether it will reduce CDI infection rates is not understood. Since treatment does not
409 reliably clear *C. difficile* in significant proportion of patients with CDI, antimicrobial
410 clearance of carriage is also likely to be ineffective as well.

411

412 9. Much of the debate seems to be about the potential for false-positive results for *C.*
413 *difficile* infection. What are the consequences of administering antibiotics to treat *C.*
414 *difficile* infection to patients who are colonized, but not infected, with *C. difficile*?

415 **Fang-** Administering antibiotics to asymptomatic colonized patients will not provide a clinical
416 benefit and will disrupt the host microbiota. The use of unnecessary antibiotics can also
417 promote the emergence of antibiotic-resistant organisms such as VRE (vancomycin-resistant
418 enterococci) (70).

419 **Polage and Wilcox-** Antibiotic treatment for CDI is not benign. Metronidazole and vancomycin
420 increase the risk of colonization and infection with multi-drug resistant organisms and
421 promote rebound overgrowth of *C. difficile* in colonized patients after antibiotic
422 discontinuation, which can lead to prolonged shedding or active infection (CDI) (71-73).
423 Reflexive treatment of patients with false-positive results for CDI can also lead to delayed
424 recognition of outbreaks (e.g., norovirus) or alternative diagnoses (e.g., medication-induced
425 diarrhea, ischemic colitis), and treatment failure (67-69). In the near future, antibiotic use in
426 hospitals will be reported publically and hospitals will be mandated to implement
427 antimicrobial stewardship programs to improve antibiotic use, creating additional incentives
428 for hospitals to curb excessive/unnecessary antibiotic use. Thus, routine administration of
429 antibiotics to patients with false-positive results for CDI has significant negative
430 consequences for patients and hospitals.

431

432

433

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