Reply to Tenover et al., “Guidelines Support the Value of Stand-Alone Nucleic Acid Amplification Tests for *Clostridioides (Clostridium) difficile* Infection”

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In a letter to the editor (1) in response to Sandlund and Wilcox (2), Tenover et al. suggest that on-label testing with nucleic acid amplification tests (NAATs) for the diagnosis of *Clostridioides difficile* infection (CDI) be discussed to put toxin testing in perspective. The authors argue that guidelines from the Infectious Diseases Society of America–Society for Healthcare Epidemiology of America (IDSA-SHEA) (3), the American Society for Microbiology (ASM) (4), and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) (5), “all make it clear that NAATs play an essential role in the laboratory diagnosis of CDI.” This statement is potentially misleading. First, the ESCMID guidelines state that “using NAAT as a stand-alone test and relying on clinical symptoms to discern patients with CDI from asymptomatic carriers is not an optimal approach: patients colonized by a toxigenic *C. difficile* strain may very well develop diarrhea due to other causes (5).” Second, the systematic review from ASM evaluated performance compared to the detection of *C. difficile* organism/toxin/toxin gene and not to clinical diagnosis (4). However, this systematic review evaluated only testing methods and algorithms that include NAAT, and it excluded key studies that have demonstrated the clinical value of toxin-based testing (6, 7). Tenover et al. claim that ASM guidelines endorse “a role for stand-alone NAATs for CDI,” while the ASM authors state that the use of NAAT alone is recommended best practice “for the detection of the *C. difficile* toxin gene or organism” (4). The two statements are certainly not synonymous. Third, the IDSA-SHEA guidelines do recommend that NAAT can be used alone, but only when there are preagreed institutional criteria for patient stool submission (weak recommendation, low quality of evidence). These guidelines also clearly recommend an algorithmic approach to CDI diagnosis that includes toxin testing. The clinical criteria, unexplained and new onset of ≥ 3 unformed stools in 24 hours in patients not receiving laxatives, are discussed in the guidelines: “some of these conditions and interventions associated with diarrhea in their own right, [. . .], have been shown to have increased risk of CDI. So, in practice it is difficult to exclude the possibility of CDI on clinical grounds alone in a patient with new-onset or worsened diarrhea (3).”

Tenover et al. agree that using NAAT for the diagnosis of CDI leads to overdiagnosis, but only if the clinical criteria for testing are not met. The authors further argue that any diagnostic *C. difficile* assay can be positive in asymptomatic carriers. In a recent study, it was shown that the proportion of CDI overdiagnosis was over three times higher in NAAT⁺/toxin[−] patients than in NAAT⁺/toxin⁺ patients when an ultrasensitive toxin assay was used for CDI diagnosis, in an institution where rigorous stool submission criteria were recently successfully set in place (8, 9). CDI is a toxin-mediated disease and, although it has been known for decades that toxin-producing strains can be present in asymptomatic carriers (10, 11), presence of toxins better correlates with disease and with outcome than does the presence of toxin genes (6–8). Hospital-onset diarrhea is

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a common condition and, importantly, a recent large study showed that the majority of cases (85%) have multiple possible causes (median, 3; interquartile range [IQR], 2 to 5) (12). Thus, reliance on NAAT alone for the diagnosis of CDI will still lead to overdiagnosis even if clinical criteria are used to guide who/when to test.

A number of NAAT qualities are highlighted in the letter, namely, speed, sensitivity, high negative predictive value, and cost effectiveness when used appropriately (although the latter can be debated). The authors have, rightfully, left out high clinical specificity and high positive predictive value, both critical components of any diagnostic test. Surprisingly, Tenover et al. close their letter with the statement “we simply note that three recent guidelines support the value of NAATs for diagnosing CDI, while none indicate a role for ultrasensitive toxin tests.” Notably, however, an ultrasensitive toxin test was not commercially available at the time of publication of these guidelines, making such a recommendation impossible.

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