**Reply to Comment Letter**

Johanna Sandlund[1], Mark H Wilcox[2]

[1]Singulex, Inc

[2]Leeds Teaching Hospitals NHS Trust and University of Leeds

**To the Editor**

In a Letter to the Editor, Tenover *et al* suggest that on-label testing with nucleic acid amplification tests (NAATs) for the diagnosis of *Clostridioides difficile* infection (CDI) is discussed, to put toxin testing in perspective. The authors argue that guidelines from the Infectious Diseases Society of American- Society for Healthcare Epidemiology of America (IDSA-SHEA)(1), the American Society for Microbiology (ASM) (2), and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) (3), "all make it clear that NAATs play an essential role in the laboratory diagnosis of CDI." This statement is potentially misleading. Firstly, 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 (3)." Secondly, the systematic review from ASM evaluated performance compared to detection of *C. difficile* organism/toxin/toxin and not clinical diagnosis (2). However, this systematic review somewhat disingenuously evaluated only testing methods and algorithms including NAAT, and excluded key studies that have demonstrated the clinical value of toxin based testing (4, 5). Tenover *et al* claim that ASM guidelines "endorses 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 (2)." The two statements are certainly not synonymous. Thirdly, the IDSA-SHEA guidelines do

recommend that NAAT can be used alone, but only when there are pre-agreed institutional criteria for patient stool submission (weak recommendation, low quality of evidence). These guidelines also clearly recommend an algorithm approach to CDI diagnosis that includes toxin testing. The clinical criteria, unexplained and new-onset ≥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 (1)."

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- than in NAAT+/toxin+ patients when an ultrasensitive toxin assays was used for CDI diagnosis, in an institution where rigorous stool-submission criteria were recently successfully set in place (6, 7). CDI is a toxin-mediated disease and, although it has been known for decades that toxin-producing strains can be present in asymptomatic carriers (8, 9), presence of toxins better correlate with disease *and* outcome than presence of toxin genes (4–6). Hospital-onset diarrhea is a common condition and importantly a recent large study showed that the majority (85%) have multiple possible causes (median 3; IQR 2-5) (10). 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: speed, sensitivity, high negative predictive value, and cost-effectiveness when used appropriately (although the latter can be debated). The authors

have, rightfully so, 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 of '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.

## References


1. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. 2018. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis Off Publ Infect Dis Soc Am.

2. Kraft CS, Parrott JS, Cornish NE, Rubinstein ML, Weissfeld AS, McNult P, Nachamkin I, Humphries RM, Kirn TJ, Dien Bard J, Lutgring JD, Gullett JC, Bittencourt CE, Benson S, Bobenchik AM, Sautter RL, Baselski V, Atlas MC, Marlowe EM, Miller NS, Fischer M, Richter SS, Gilligan P, Snyder JW. 2019. A Laboratory Medicine Best Practices Systematic Review and Meta-analysis of Nucleic Acid Amplification Tests (NAATs) and Algorithms Including NAATs for the Diagnosis of Clostridioides (Clostridium) difficile in Adults. Clin Microbiol Rev 32.

3. Crobach MJT, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, Wilcox MH, Kuijper EJ. 2016. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 22 Suppl 4:S63-81.

4. Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, O'Connor L, Oakley SJ, Pope CF, Wren MW, Shetty NP, Crook DW, Wilcox MH. 2013. Differences in outcome according to Clostridium difficile testing method: a prospective multicentre diagnostic validation study of C difficile infection. Lancet Infect Dis 13:936–945.

5. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang Y-W, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. 2015.

74 Overdiagnosis of Clostridium difficile Infection in the Molecular Test Era. JAMA Intern Med
75 175:1792–1801.

76 6. Sandlund J, Estis J, Katzenbach P. 2019. Evaluation of the Singulex Clarity C. diff Toxins A/B Assay
77 for Diagnosis of Clostridioides difficile Infection. ASM Microbe. 2019.

78 7. Chow S-K, Naderpour A, Van Enk J. 2018. It Is Not about the Assay: Preanalytical Screening Is the
79 Key to Reducing *Clostridioides difficile* Infection. J Clin Microbiol 57.

80 8. Kyne L, Warny M, Qamar A, Kelly CP. 2000. Asymptomatic carriage of Clostridium difficile and
81 serum levels of IgG antibody against toxin A. N Engl J Med 342:390–397.

82 9. Kyne L, Warny M, Qamar A, Kelly CP. 2001. Association between antibody response to toxin A and
83 protection against recurrent Clostridium difficile diarrhoea. Lancet Lond Engl 357:189–193.

84 10. Mawer D, Byrne F, Drake S, Brown C, Prescott A, Warne B, Bousfield R, Skittrall JP, Ramsay I,
85 Somasunderam D, Bevan M, Coslett J, Rao J, Stanley P, Kennedy A, Dobson R, Long S, Obisanya T,
86 Esmailji T, Petridou C, Saeed K, Brechany K, Davis-Blue K, O'Horan H, Wake B, Martin J,
87 Featherstone J, Hall C, Allen J, Johnson G, Hornigold C, Amir N, Henderson K, McClements C, Liew I,
88 Deshpande A, Vink E, Trigg D, Guilfoyle J, Scarborough M, Scarborough C, Wong THN, Walker T,
89 Fawcett N, Morris G, Tomlin K, Grix C, O'Cofaigh E, McCaffrey D, Cooper M, Corbett K, French K,
90 Harper S, Hayward C, Reid M, Whatley V, Winfield J, Hoque S, Kelly L, King I, Bradley A, McCullagh
91 B, Hibberd C, Merron M, McCabe C, Horridge S, Taylor J, Koo S, Elsanousi F, Saunders R, Lim F,
92 Bond A, Stone S, Milligan ID, Mack DJF, Nagar A, West RM, Wilcox MH, Kirby A, Sandoe J a. T. 2019.
93 Cross-sectional study of the prevalence, causes and management of hospital-onset diarrhoea. J
94 Hosp Infect.