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PCR cycle threshold to assess a diagnostic stewardship intervention for *C. difficile* testing

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Dear Editor

We read with interest the article by Kamboj et al., who demonstrated that low *Clostridium difficile* real-time polymerase chain reaction (PCR) cycle thresholds of detection (C_T) were predictive of toxin enzyme immunoassay positivity and disease severity in oncology patients who showed a positive *C. difficile* nucleic acid amplification test (NAAT) result.[1] These findings are consistent with those reported in other studies that found *C. difficile* PCR C_T (i.e., ≤ 26.0–28.0) may be similar to the results obtained from the cell cytotoxicity neutralization assay (CCNA) and superior to those obtained from toxin enzyme immunoassay in differentiating clinical *C. difficile* infection (CDI).[2–5]

We previously reported the use of a computerized clinical decision support (CCDS) tool that led to significantly reduced NAAT testing and National Healthcare Safety Network (NHSN) surveillance CDI events in our institution.[6] On the basis of the report by Kamobj et al., we sought to determine whether C_T data contributed to the identification of patients with lower probability of the disease.

Positive GeneXpert (Cepheid, Sunnyvale, CA) NAAT results were analyzed retrospectively between January 2014 and June 2018. C_T values obtained from tests that were ordered appropriately, according to the CCDS tool, were compared with those obtained from tests categorized as inappropriate. Inappropriate orders were defined as patients identified by the provider through the CCDS tool (post-CCDS) as lacking diarrhea or signs/symptoms of CDI

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Conflict of interests

None to declare.

or automatically flagged as a duplicate test (pre- or post-CCDS). A very high C_T value was defined as > 30.85, which is shown to have a 98.7% negative predictive value of a negative CCNA and toxin EIA, and thus, it likely reflects colonization with low organism burden.[4]

We found that C_T values were significantly higher in the inappropriate test group than in the appropriate test group (median: 26.7 versus 24.8 cycles, Table 1). The strongest predictor of an increased C_T value was a duplicate of a negative test. Fig. 1 demonstrates that C_T values were increased in the inappropriate test group, with a clustering of very high C_T results.

These results support the use of our current CCDS-based strategy. It is difficult to ascertain whether the result of 22.2% of very high C_T values (>30.85) obtained from the appropriate test (compared to the result of 34.0% of very high C_T values obtained from the inappropriate test) is acceptable or not. We hypothesize that refinement of the CCDS may further reduce the proportion of tests with very high C_T values. In addition, it should be noted that 35% of C_T values categorized as inappropriate (excluding duplicates of positives) were < 26.0, thus suggesting that the patients were mis-classified as being at a low pretest probability for the disease. We feel that this supports the use of CCDS tools for diagnostic guidance during test ordering, while allowing clinicians to bypass the tool and order tests on the basis of their clinical judgment.

Although the absolute difference in the median C_T value between groups is relatively small, this likely reflects the prevalence of *C. difficile* colonization described among hospitalized patients (~4–29%) and the fact that colonized patients outnumber infected patients as 5 to 1.[7] Although we have not validated C_T values with CCNA at our institution, we found a similar association between C_T and toxin EIA described by Kamboj et al. and others, using a small (70 positive NAAT samples) set of historical internal validation samples (data not shown).

Considering the gold standard among *C. difficile* diagnostics, the CCNA assay is technically complex and labor intensive and has a slow turnaround time, thus making it impractical for routine clinical use. Unfortunately, *C. difficile* EIAs lack sensitivity. For these reasons, > 70% of hospitals currently use NAAT for the diagnosis of CDI.[8] The GeneXpert *C. difficile* PCR assay is highly sensitive at the manufacturer-set maximum C_T (≤ 37.0), with an estimated detection limit of 1657 colony-forming units; however, a positive NAAT result alone may overdiagnose CDI up to half of the time.[2]

Analysis of C_T may offer a means to tailor *C. difficile* NAAT sensitivity and specificity according to various patient populations and levels of risk by modulating the C_T along a receiver operator characteristic curve. C_T also allows valuable feedback for diagnostic stewards, as we have shown. Validation of C_T for diagnostic and diagnostic stewardship purposes requires further research in various clinical settings before its clinical use can be widely applied.

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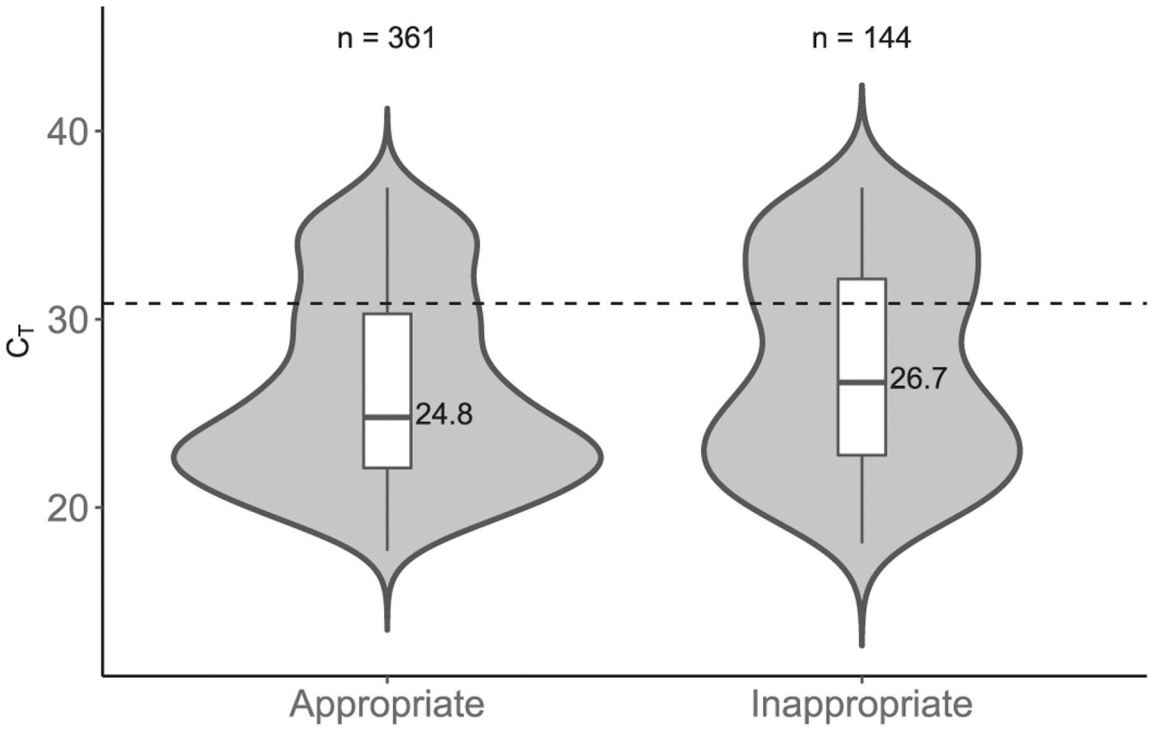

Fig. 1.

Violin and box plots comparing C_T values between appropriate and inappropriate positive *C. difficile* NAATs. The dotted line depicts very high threshold = 30.85.

Table 1

C_T values by order appropriateness

		Appropriate		Inappropriate		
	Total	***n* (%)**	**Median C_T (IQR)**	***n* (%)**	**Median C_T (IQR)**	***P***
CCDS question						
Presence of diarrhea? (Appropriate Response = “Yes”)	460	453 (98.5%)	24.9 (22.1–30.4)	7 (1.5%)	25.0 (22.2–29.9)	.847
Signs/Symptoms of CDI? (Appropriate Response = “Yes”)	460	375 (81.5%)	24.9 (22.1–30.3)	85 (18.5%)	25.6 (22.3–31.2)	.393
Duplicate test *						
Duplicate of positive	1839	1799 (97.8%)	25.4 (22.4–30.7)	40 (2.2%)	27.3 (23.5–32.4)	.087
Duplicate of negative	1839	1825 (99.2%)	25.4 (22.4–30.7)	14 (0.8%)	31.6 (27.7–34.4)	.003
Inappropriate CCDS response or duplicate Test **	505	361 (71.5%)	24.8 (22.1–30.3)	144 (28.5%)	26.7 (22.8–32.2)	.023

P values were obtained by the Mann–Whitney U test. Duplicate of negative is defined as a negative result within 3 days of a previous negative result. Duplicate of positive is defined as a positive result within 14 days of a previous positive result.

* Three of the duplicate of negative and six of the duplicate of positive tests were performed post-CCDS implementation.

** Compared to all appropriate positive tests post-CCDS implementation.