Clostridioides difficile PCR Cycle Threshold to Determine Toxin Positivity Status

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Background

- There is no universal standard regarding testing for *C. difficile* infection (CDI).
- Many clinical laboratories rely on polymerase chain reaction (PCR) as the sole test for diagnosis of CDI which can result in the overdiagnosis of CDI.
- A 2-step testing algorithm using PCR and *C. difficile* toxin immunoassay can increase diagnostic certainty.

Objective

The objectives of this study were to determine if *C. difficile* (CD) PCR cycle threshold (Ct) could be used to predict the presence of *C. difficile* toxin and determine the clinical outcomes of those deemed to be toxin positive by PCR cycle threshold.

Methods

- Characteristics and outcomes of 148 consecutive patients who tested positive for CD by PCR (Xpert CD, Cepheid) between October 2019 and December 2021 at one VA Hospital where reflex toxin testing (Cdiff Quik Check Complete, Alere/TechLab) was performed after positive PCR result.
- Baseline characteristics, initial laboratory data, treatment, clinical outcomes, and PCR Ct were collected and compared.
- Determination of CDI or CD colonization was made after chart review by an Infectious Disease physician prior to review of PCR Ct.
- CDI diagnosis required documentation of the following clinical symptoms:
  - ≥3 unformed bowel movements per day
  - Description of diarrhea by care team (Bristol stool scale ≥4)
- Consideration of CD colonization required either documentation of colonization by the care team or a reasonable alternative explanation of diarrhea (E.g., receipt of laxatives within 48 hours of diarrhea onset).
- A PCR Ct of ≤32.2 (75th percentile) was set as the cutoff Ct based on the optimal receiver operator characteristic curve threshold.

Results

Demographics and Clinical Characteristics

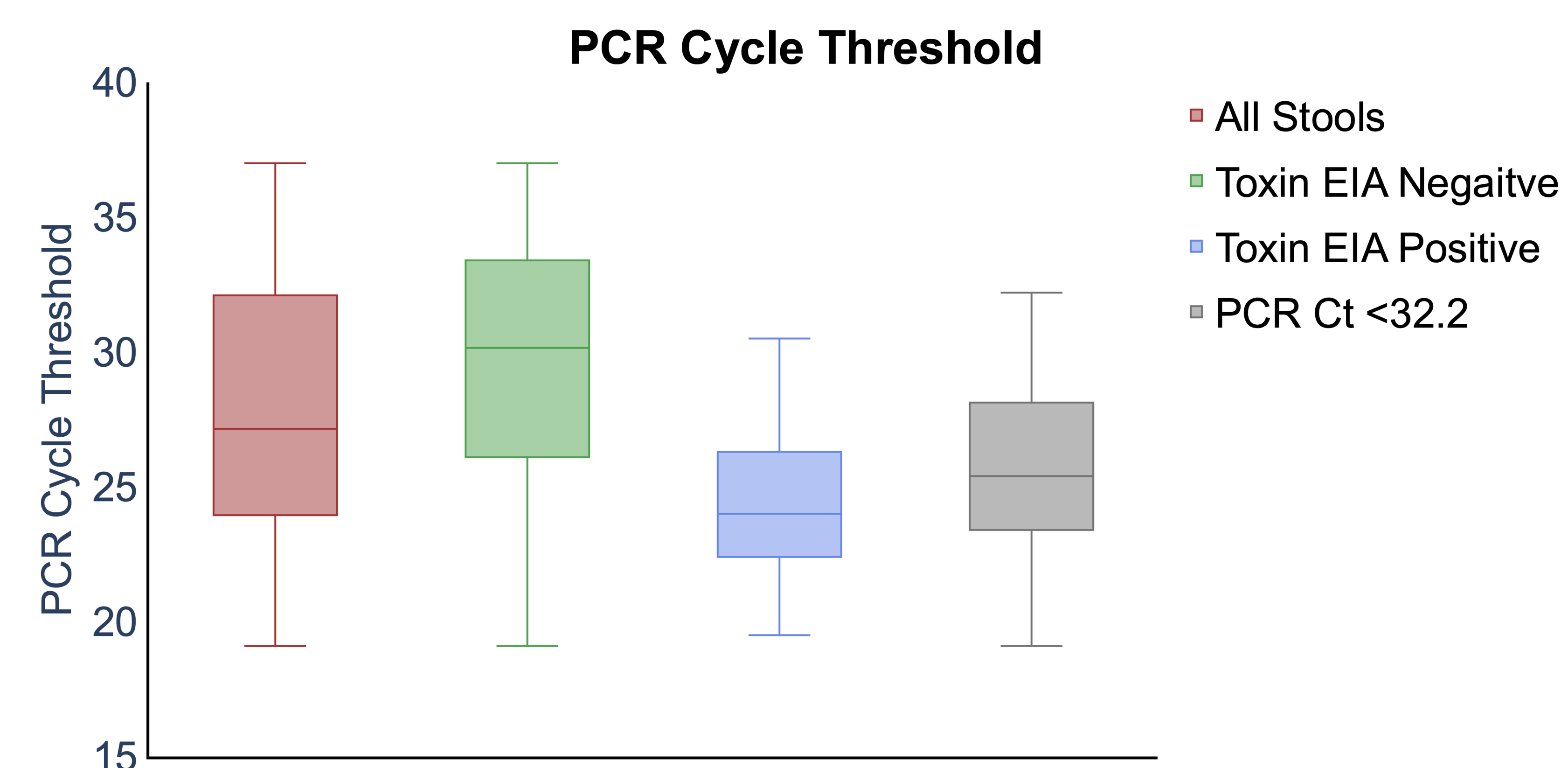|	All Stools (n=148)	Toxin Negative (n=90)	Toxin Positive (n=58)	p value	Ct >32.2 (n=35)	Ct ≤32.2 (n=113)	p value
Male Sex [%]	142 (96%)	85 (94%)	57 (98%)	0.40	34 (97%)	108 (96%)	1.00
Age ≥ 65 [%]	118 (80%)	68 (76%)	50 (86%)	0.14	29 (83%)	89 (79%)	0.81
History of CDI [%]	50 (34%)	24 (26%)	27 (47%)	0.02	7 (20%)	43 (38%)	0.10
Diarrhea [%]	137 (93%)	80 (89%)	57 (98%)	0.05	27 (77%)	110 (97%)	<0.01
Laxative Use [%]	39 (26%)	29 (32%)	10 (17%)	0.06	7 (20%)	32 (38%)	0.39
Abdominal Pain [%]	43 (29%)	22 (24%)	21 (36%)	0.14	7 (20%)	36 (32%)	0.21
Temp >100.4 ºF [%]	15 (10%)	4 (4%)	11 (19%)	0.01	2 (5%)	13 (12%)	0.52
WBC (K/µL) [95% CI]	10.00 (±1.0)	8.8 (±0.9)	11.8 (±1.9)	<0.01	7.9 (±1.6)	10.6 (±1.1)	0.02
Creatinine (mg/dL) [95% CI]	1.83 (±0.3)	1.7 (±0.5)	2.0 (±0.5)	0.44	1.7 (±0.5)	1.9 (±0.4)	0.46
Albumin (g/dL) [95% CI]	2.63 (±0.1)	2.8 (±0.2)	2.5 (±0.1)	0.05	2.7 (±0.4)	2.6 (±0.1)	0.62

Clinical Outcomes

	All Stools (n=148)	Toxin Negative (n=90)	Toxin Positive (n=58)	p value	Ct >32.2 (n=35)	Ct ≤32.2 (n=113)	p value
CDI [%]	108 (73%)	53 (59%)	55 (95%)	<0.01	18 (51%)	90 (80%)	<0.01
Treatment for CDI [%]	121 (82%)	64 (71%)	57 (98%)	<0.01	27 (77%)	94 (83%)	0.46
CDI Recurrence 30 days [%]	15 (10%)	6 (7%)	9 (16%)	0.10	1 (3%)	14 (12%)	0.12
CDI Recurrence 90 days [%]	25 (17%)	12 (13%)	13 (22%)	0.18	2 (6%)	23 (20%)	0.06
PCR Ct [95% CI]	27.87 (±0.76)	29.97 (±0.93)	24.61 (±0.79)	<0.01	34.60 (±0.55)	25.78 (±0.58)	<0.01

Receiver Operator Characteristic Curve

(AUC=0.8370)
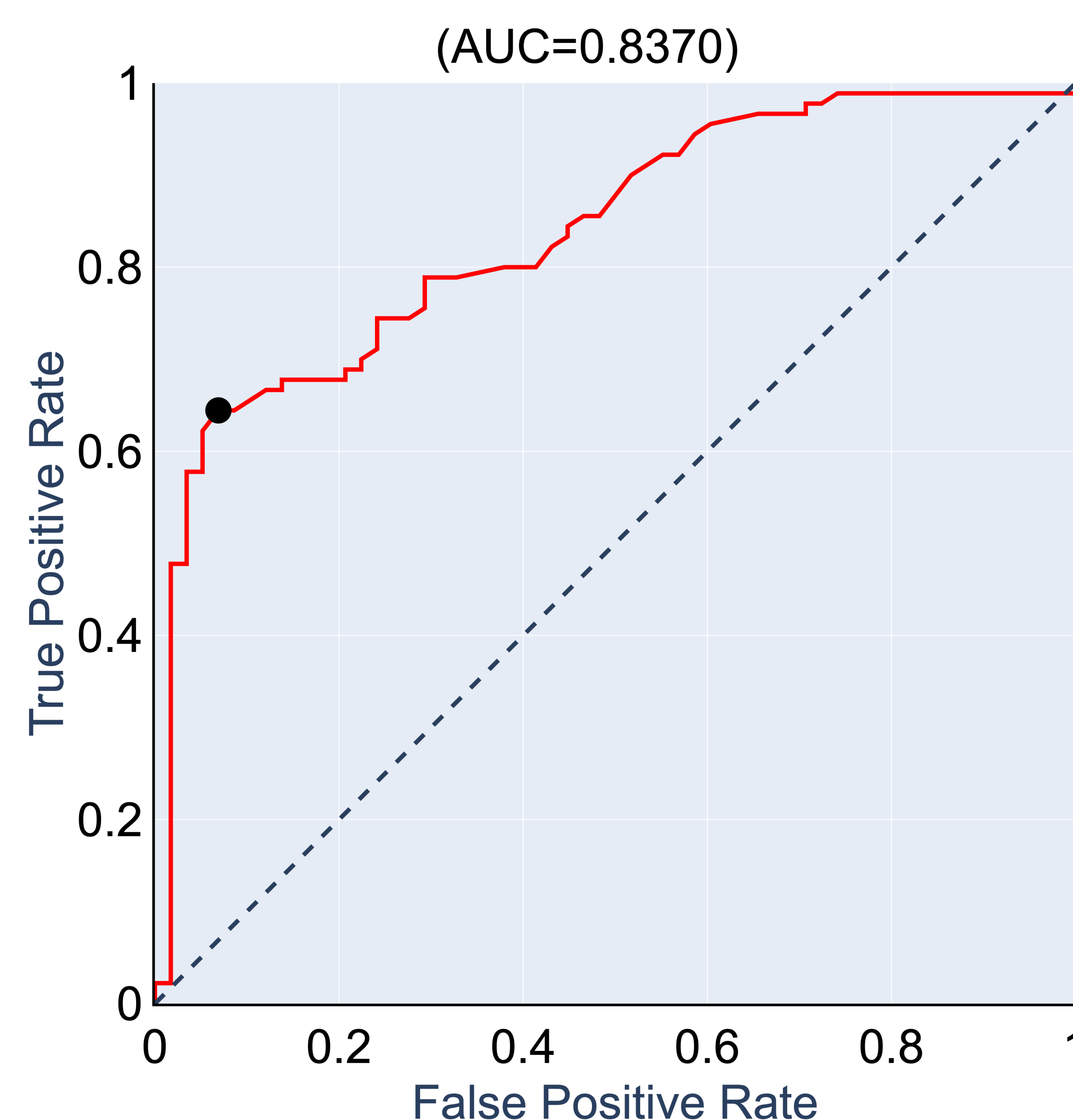
Boxplot PCR Cycle Threshold

PCR Cycle Threshold

- All Stools
- Toxin EIA Negaitve
- Toxin EIA Positive
- PCR Ct <32.2

Conclusions

In a setting where a CD toxin assay is not readily available, a CD stool PCR cycle threshold could be used to predict toxin status. In our study, a cycle threshold of ≤32.2 predicted both toxin-positivity and CDI.