

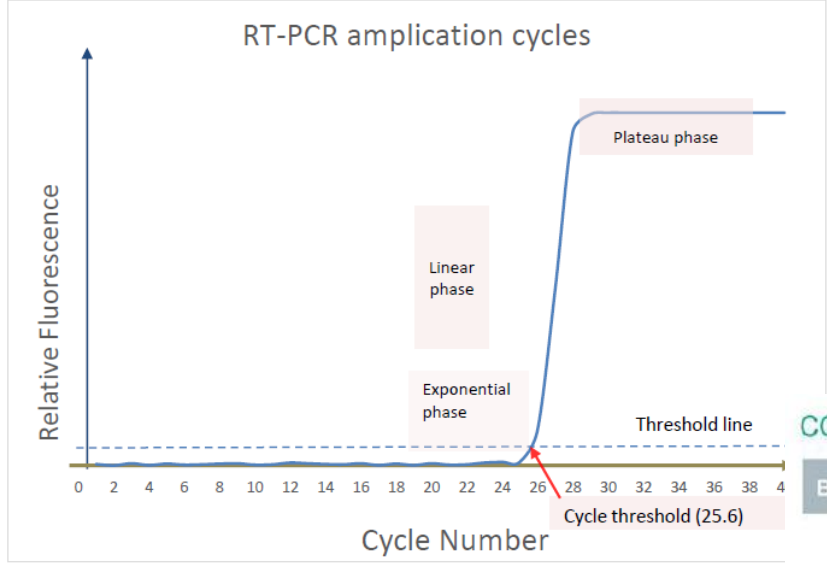
CLINICAL UTILITY OF CYCLE THRESHOLD VALUES: LESSONS LEARNED FROM COVID- 19 AND APPLICATION IN THE FUTURE

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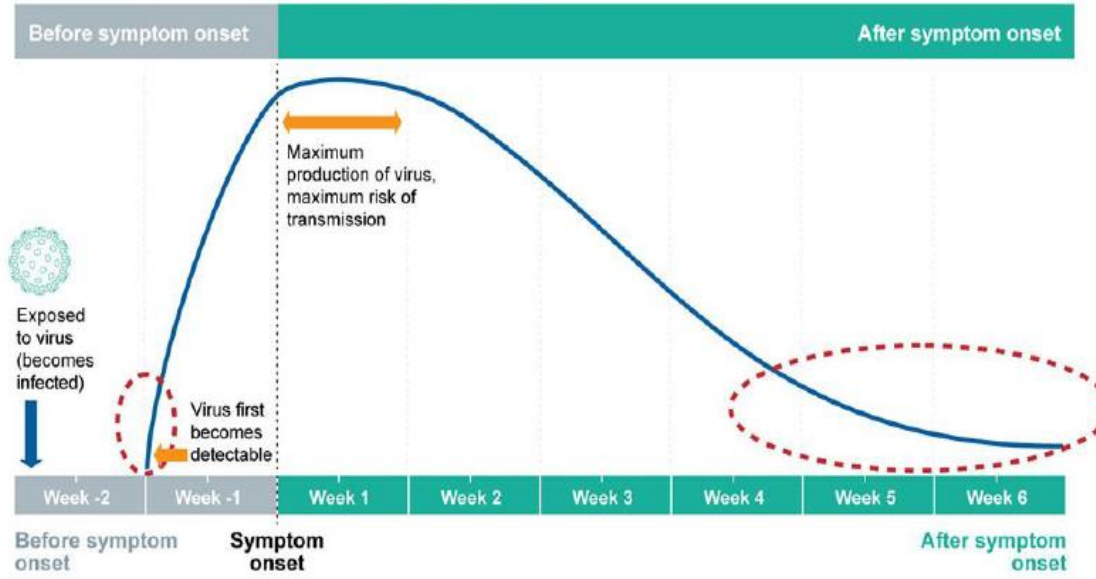
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THE CT

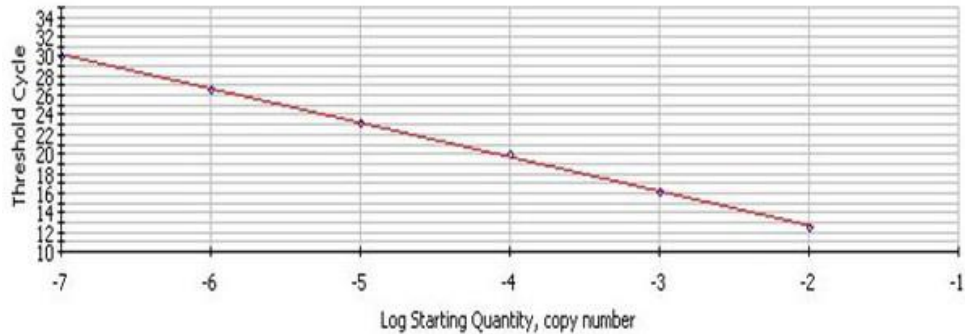
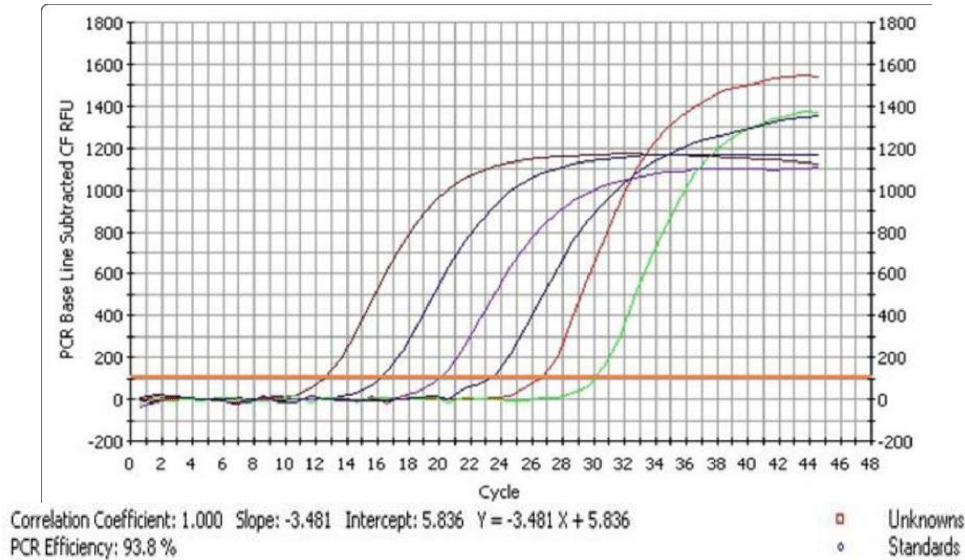


COVID-19 symptom onset schematic diagram



Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR. 2020. Public Health England.

CORRELATING CT WITH VIRAL QUANTITATION



- A standard curve is generated using known quantitative standards
- A best fit line allows linear equation ($Y=mx+b$) to determine the viral load by solving for X
 - Y= the Ct value of the patient PCR value
 - X=viral load, as established by the standard curve

IDSA and AMP joint statement on the use of SARS-CoV-2 PCR cycle threshold (Ct) values for clinical decision-making



Updated March 12, 2021

- **Relationship between Ct values and SARS CoV-2 viral load**

- In general, low Ct values indicate a higher viral density and high Ct values generally indicate a lower viral.
- Qualitative real-time PCR tests are not designed to provide a quantitative or semi-quantitative measurement of nucleic acid in a sample.
- **This is because qualitative test Ct values are not normalized to standardized controls of known concentration.**
- Qualitative assays are not optimized to have a linear relationship between the Ct value and the concentration of target in the specimen, which may disproportionately impact the reliability of Ct values obtained from samples containing either high or low viral loads.

- **Factors affecting Ct values**

- Multiple variables may impact the determination of Ct values .
- As a result, Ct values may not be comparable for individual patients tested sequentially with the same method and are definitely not directly comparable across different real-time PCR tests or testing laboratories.
- The College of American Pathologists (CAP) recently reported the results of SARS CoV-2 PCR proficiency testing conducted across 700 laboratories in the US and found that the median Ct values for a control sample varied by up to 14 cycles (i.e., 4000-fold) across platforms and reproducibility within the same instrument differed by a median of 3 cycles (i.e., 10-fold) [1].
- It is also important to note that clinical labs often perform SARS-CoV-2 testing using a variety of different tests and testing platforms. .

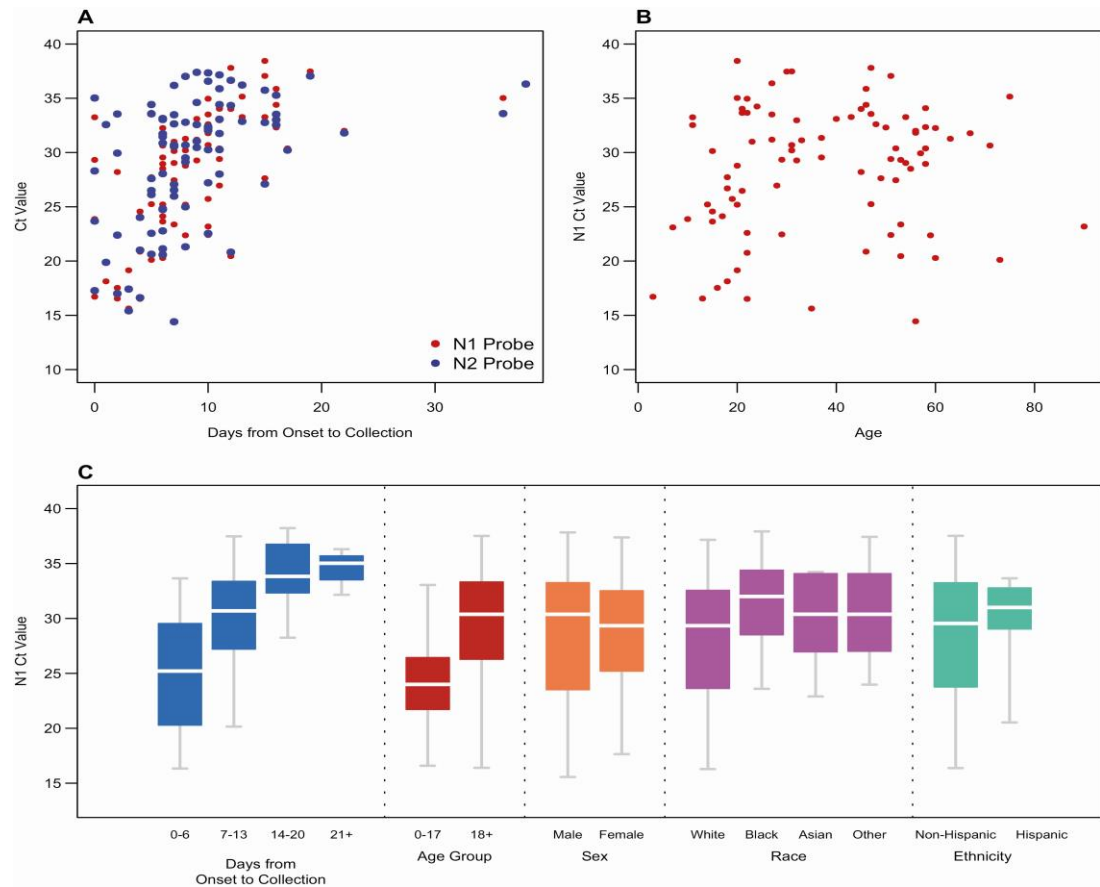
IDSA and AMP joint statement on the use of SARS-CoV-2 PCR cycle threshold (Ct) values for clinical decision-making



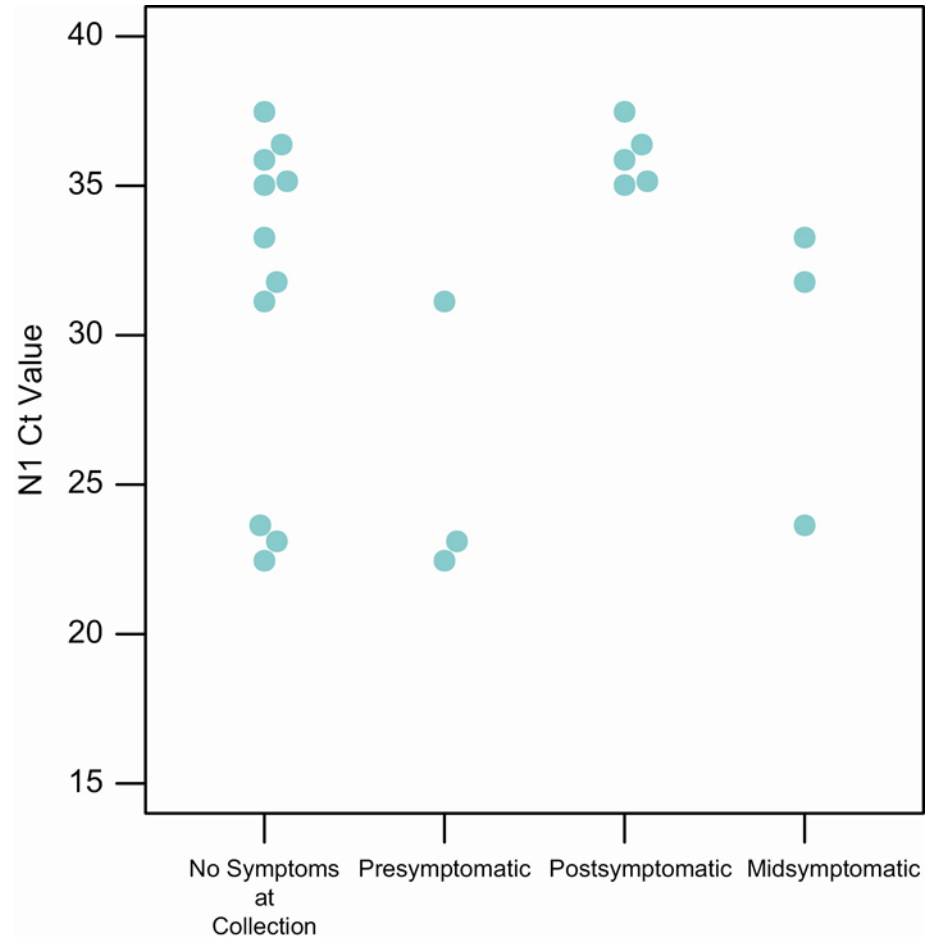
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- **Do Ct values predict disease severity?**
 - Given the wide variability of Ct values both across and within PCR platforms, it is not possible to identify a universal threshold of prognostic value.
- **Can Ct values identify which individuals are most infectious to others?**
 - At this time, additional data are needed regarding when an individual is no longer infectious before Ct values alone can be used to inform decision making.
- **Take home messages**
 - Although there is a relative relationship between Ct values and the amount of virus in a clinical specimen, Ct values generated by qualitative PCR tests should not be considered quantitative measures of viral load.
 - Due to the variety of factors known to impact Ct values, caution is advised when applying published correlations of Ct values with disease severity or as a predictor of active infection and hence transmissibility.
 - At the current time, routine use of Ct values to inform clinical decision making is not advised. Development of a quantitative SARS-CoV-2 real-time PCR assay may overcome some of the limitations outlined above.

CORRELATES OF CT VALUES FROM PARTICIPANTS IN COVID-19 HOUSEHOLD TRANSMISSION INVESTIGATIONS: UTAH AND WISCONSIN



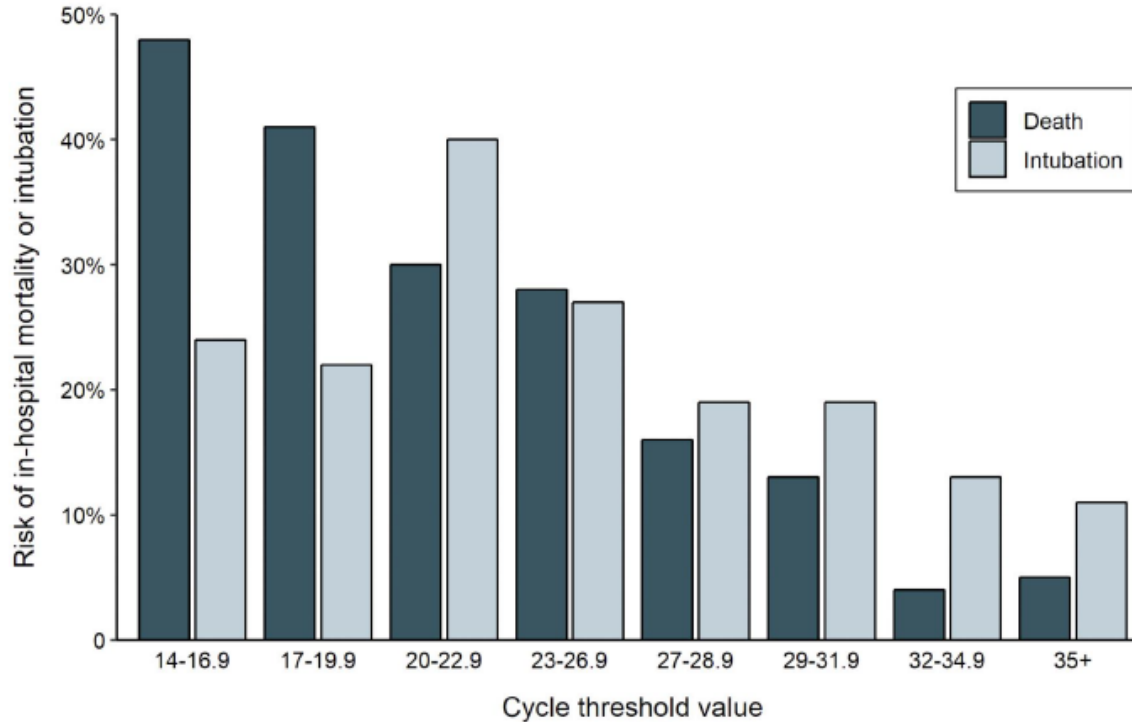
CT VALUES AMONG PARTICIPANTS REPORTING NO SYMPTOMS AT THE TIME OF POSITIVE NP



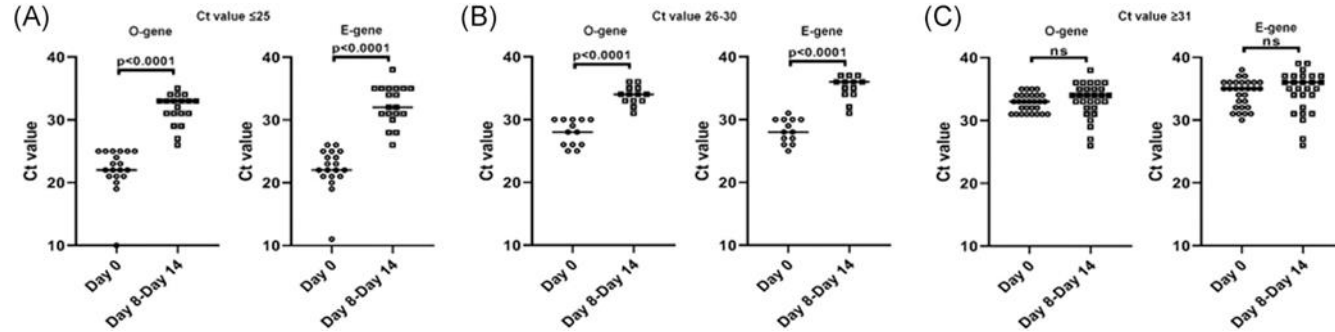
IS THERE A CORRELATION BETWEEN CT VALUE AND
OUTCOMES?



CORRELATION BETWEEN CT AND POOR OUTCOMES?



CT AS A MARKER FOR ISOLATION



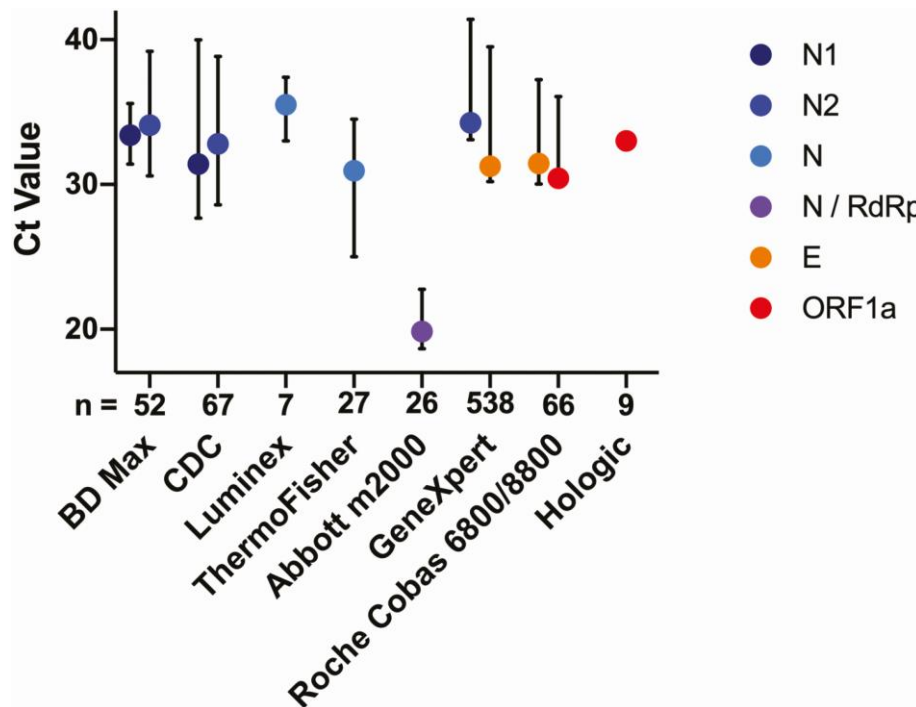
WHAT CONSIDERATIONS ARE THERE FOR USING CT
VALUES IN PATIENT CARE?



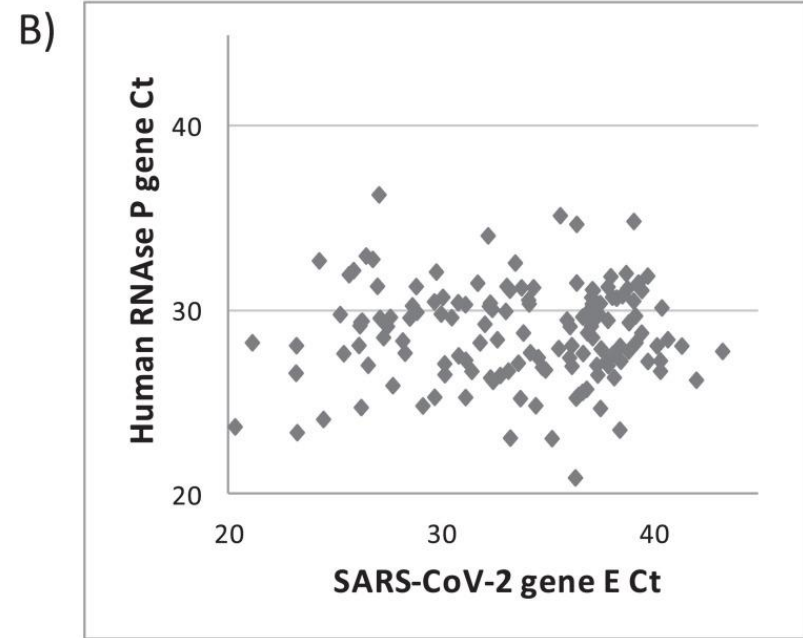
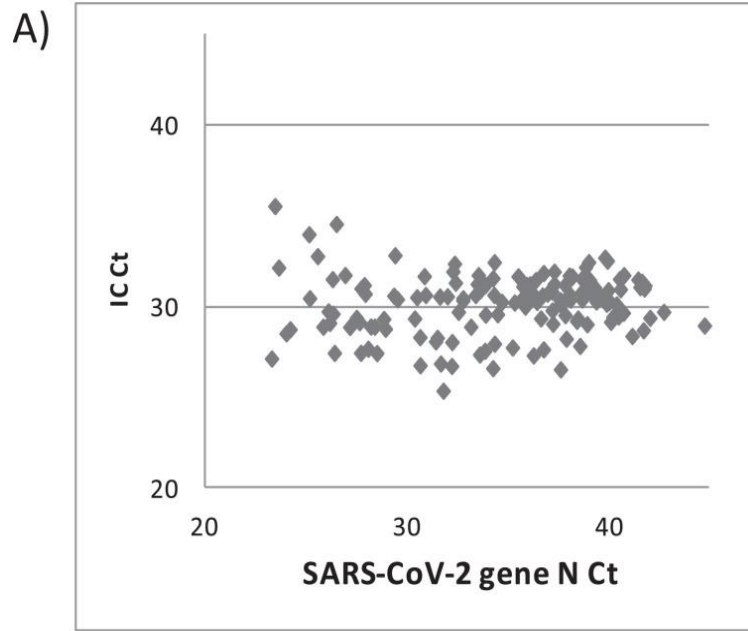
IMPORTANT CONSIDERATIONS

- Specimen collection
 - Significant variation in technique among collectors
 - Transport media formula vs saline vs saliva
 - Transport media volume
 - Transport time and conditions
 - Specimen collection method – NP versus Nasal
- Controlling for Quantitation
 - Qualitative assays are designed for reliable quantitation, need to include reference standards to ensure reproducibility
 - Need to confirm precision of your real time PCR assay
- Testing
 - Significant variation in Ct between methods, labs often use multiple manufacturers tests and comparison among tests can be challenging
 - Assay targets can quantitate differently and have different limits of detection
 - Extraction platform can account for variability

CT VALUES FOR GENE TARGETS AND MANUFACTURERS FOR THE SAME BATCH OF TESTING MATERIAL



VARIATION IN CT OF THE INTERNAL CONTROL



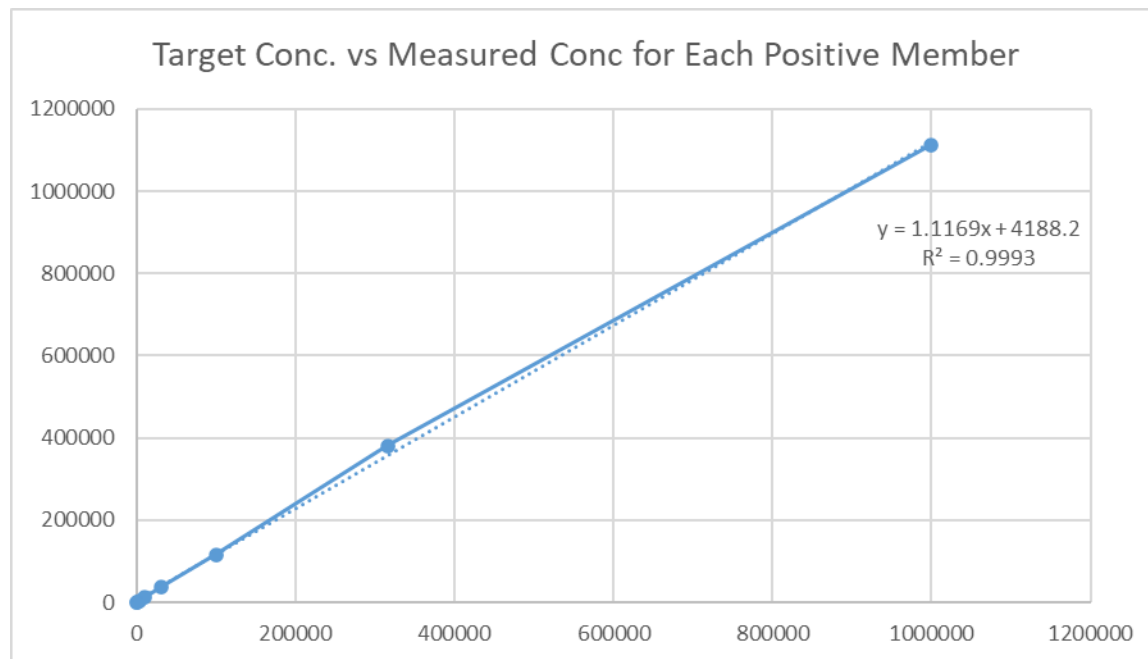
Analysis of the SARS-CoV-2 C_t values obtained using a commercial RT-qPCR assay (Vircell) in a set of clinical samples. A) C_t s of the Internal Control RNA plotted against the SARS-CoV-2 N gene C_t s B). C_t s of the human RNase P plotted against the SARS-CoV-2 E gene C_t s

CAN LABORATORIES PROVIDE BETTER DATA THAN CT FOR QUANTITATION?

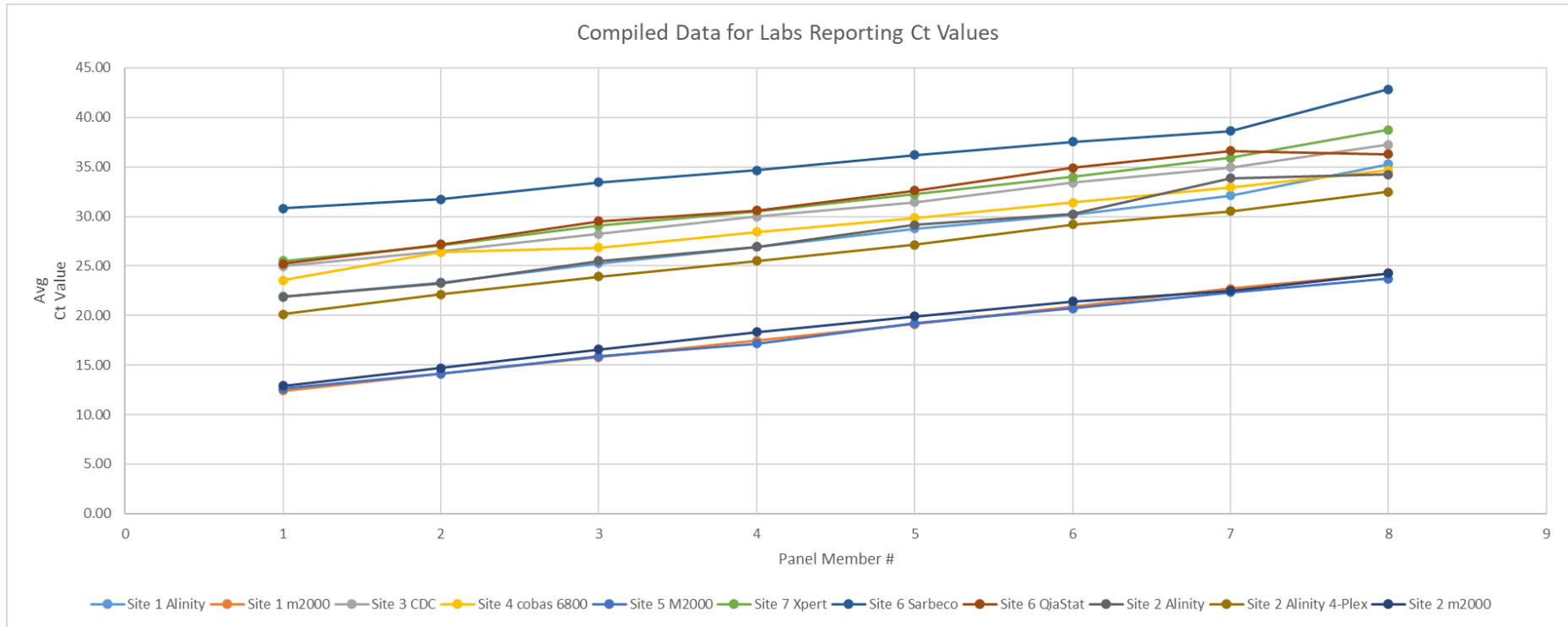
- Move from semi-quantitative RT-PCR by “reporting” Ct values, generate a standard curve and report copies/mL
 - Acknowledge limitations
- Developed a set of calibrators ranging from 300-1,000,000 copies/mL
 - Assess linearity of panel using qPCR and dPCR
- Evaluate panel across multiple platforms in 7 labs with geographical diversity across US using both Ct and copies/mL

DEVELOPING A PANEL OF CALIBRATORS TO BUILD A QPCR

Target	Measured
1000000	1113414
316000	381523
100000	116285
31600	38577
10000	11426
3160	3651
1000	1215
316	393

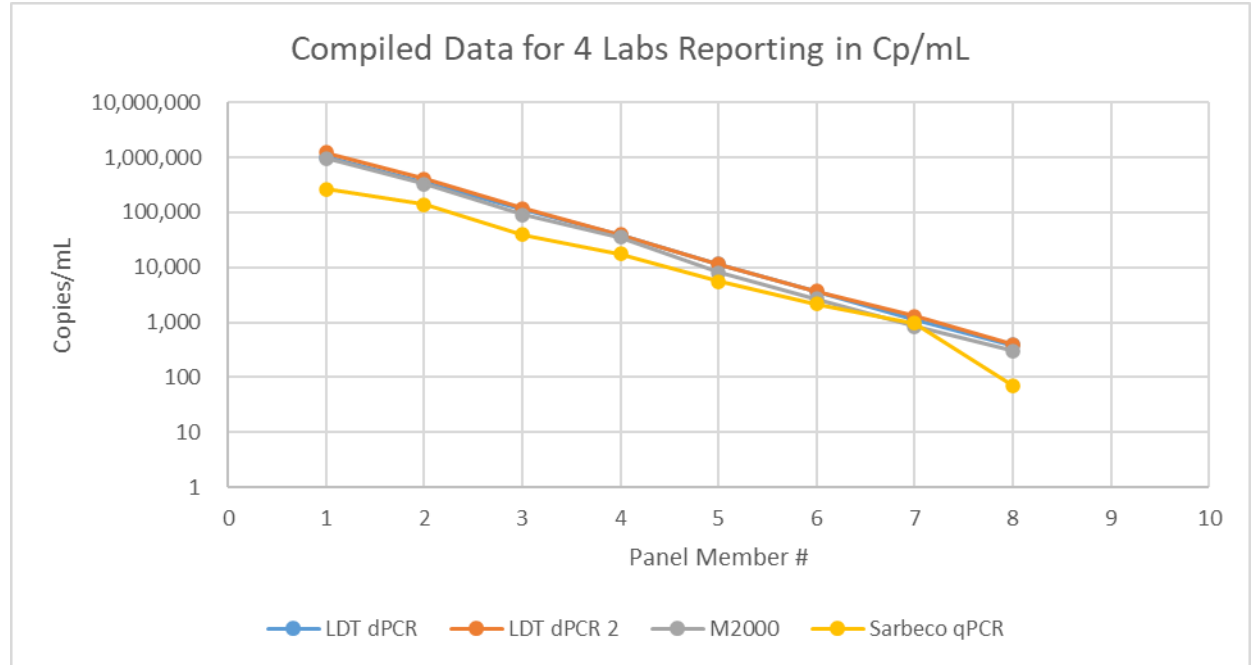


ASSESSING CT ACROSS MULTIPLE PLATFORMS



DEVELOPING A SET OF QUANTITATIVE CALIBRATORS

- Calibrators run at 4 independent laboratories
- Methods included:
 - 2 digital PCR labs
 - M2000
 - Sarbeco qPCR



THE PANDEMIC IS OVER! WHAT ABOUT OTHER
APPLICATIONS?



THE CASE FOR *C. DIFFICILE*

CT Value	EIA Toxin Positive ^a	Total EIA ^b	Mortality/month ^c	Mortality/3 months ^c
18	1 (100%)	1	1	1
19	4 (80%)	5	2	2
20	32 (84%)	38	7	12
21	80 (85%)	94	17	25
22	113 (80%)	140	16	29
23	119 (72%)	166	23	31
24	106 (67%)	157	27	39
25	96 (56%)	171	21	34
26	63 (50%)	125	12	19
27	52 (37%)	139	19	34
28	24 (27%)	89	7	13
29	23 (24%)	95	10	16
30	17 (20%)	83	8	16
31–37	24 (7%)	347	41	60
All	754 (46%)	1650	210	331
18–25	551 (72%)	772	113	173
26–37	203 (23%)	878	97	158

- Single center study from UK NHS
- Laboratory Methods
 - Specimens collected from patients with Bristol 5-7
 - GDH Screen (Meridian Premier)
 - NAAT (Cepheid GeneXpert)
 - Premier Toxin A and B (Meridian)
- Findings
 - Low Ct is associated with increased mortality.

THE CASE FOR *C. DIFFICILE* – CT VS SEVERITY

CT value	Sensitivity (TPF)	Specificity	FPF	PPV	NPV
20	0.008	0.998	0.002	0.750	0.544
21	0.050	0.992	0.008	0.844	0.554
22	0.163	0.975	0.025	0.848	0.581
23	0.314	0.944	0.056	0.826	0.621
24	0.472	0.891	0.109	0.784	0.667
25	0.613	0.834	0.166	0.756	0.719
26	0.733	0.751	0.249	0.713	0.770
27	0.820	0.682	0.318	0.684	0.818
28	0.883	0.585	0.415	0.642	0.856
29	0.915	0.507	0.493	0.610	0.876
30	0.948	0.426	0.574	0.582	0.907
35	0.995	0.076	0.924	0.475	0.944

- Ct of 18-21 – 72% of patients had severe/recurrent CDI
- Metronidazole failed as first line therapy in 23 out of 39 cases in patients with low Ct
- Patients with Ct of 35-37, 92% of patients demonstrated mild CDI with resolution of symptoms following metronidazole

BORDETELLA, DISEASE SEVERITY AND CT VALUES

TABLE 2. Age, PCR Ct Values, and White Blood Cell Counts in Patients With and Without Severe Pertussis

Clinical Characteristics	Severe Disease (n = 24)		No Severe Disease (n = 84)		<i>P</i>
	Median	IQR	Median	IQR	
Baseline characteristics					
Age, days	42.5	28.0–55.8	72.0	44.5–106	<0.001*
Laboratory data					
Ct value, cycle	19.0	16.5–22.0	25.5	20.0–30.0	0.002*
WBC count ($\times 10^3/\mu\text{L}$)	31.3	14.2–45.6	14.8	10.9–19.8	<0.001*

*Statistically significant.

Ct indicates cycle threshold; WBC, white blood cell.

A SYSTEMATIC REVIEW OF CT AND RESPIRATORY VIRUSES

	Influenza	Rhinovirus	HCoV	RSV
Mortality	1/3 (56 patients – mean VL higher in patients that died)	0/1		
Severity	2/6 (Ct \leq 25 associated with increased severity) ^a	3/9 (Ct \geq 35 more likely to be asymptomatic)		
Duration	1/1 (Lower Ct=longer duration of symptoms)	0/1		
ICU Admission	0/3	0/1		
Hospitalization	0/4	0/1		
Length of Stay	1/3 (Ct \leq 20 = longer stay)	1/3 (Ct \leq 20 = longer stay)		
Mono-infections			2/2 (lower Ct, more likely for infection with single virus)	2/3 (lower Ct in patients with only RSV)

SUMMARY

- Ct values are not directly comparable between assays and may not be reported by some RT-PCR platforms in use.
 - Interpreting single positive Ct values for staging infectious course, prognosis, infectivity or as an indicator of recovery must be done with context about the clinical history.
 - Generally, low Ct values may indicate higher acuity and higher infectivity
 - Generally, high Ct values may be associated with lower acuity, but clinical correlation is required
- From a laboratory perspective, Ct values should only be reported and applied for clinical interpretation and action where the linearity, limit of detection and standard quantification curves are assured.
- Application of Ct values outside of SARS-CoV-2 has been investigated, additional studies are needed.