

**Supplementary Material: Validation of High-Sensitivity SARS-CoV-2 Testing for Stool -
Towards The New Normal for Fecal Microbiota Transplantation**

SARS-CoV-2 RT-PCR Validation Study

This assay was previously validated on respiratory (upper and lower) and serum samples. Respiratory sample validation was performed by testing 20 contrived positive clinical samples (5 positive each for nasopharyngeal (NP), bronchoalveolar (BAL), sputum and serum), created by spiking a known concentration of a synthetic SARS-CoV-2 plasmid (Integrated DNA Technologies (IDT), Coralville, IA) at approximately two times the assay limit of detection (LoD) and 20 contrived non-reactive samples (5 negative each for NP, BAL, sputum and serum), as well as 18 person under investigation split clinical specimens (10 oropharyngeal, 7 NP, and 1 BAL) results of which were confirmed by a secondary clinical laboratory with 100% concordance. Next a preliminary LoD was determined by testing 10-fold serial dilutions of quantitated IDT SARS-CoV-2 plasmid material. A confirmation of the LoD was determined by testing 20 extracted replicates. The LoD was determined as the lowest concentration where $\geq 95\%$ (19/20) of the replicates were positive, which was 10 copies/ul. We performed cross reactivity testing for MERS-COV, SARS-COV, Human coronavirus HKU1, Rhinovirus, Human metapneumovirus, RSV-A, and RSV-B. Only SARS-COV cross reacted with the N3 primer (which is no longer used in our assay) ¹. No other cross reactivity was observed. This in addition to extensive specificity/exclusivity testing performed by the CDC for the primer and probe sets used ^{2,3}.

Supplementary Table 1: Nasopharyngeal Stool RT-PCR Agreement (n=31)

		Nasopharyngeal RT-PCR		
		Positive	Negative	Total
Stool RT-PCR	Positive	16	0	16
	Negative	1	14	15
	Total	17	14	31

Abbreviations: RT-PCR: reverse transcriptase polymerase chain reaction

Supplementary Table 2. NP RT-PCR positive patients

Patient ID	SARS-CoV-2 NP swab result	SARS-CoV-2 RT-PCR stool result	Day from symptom onset to NP testing	Day from symptom onset to stool testing	NP C _T value	Stool C _T value	SARS-CoV-2 serology testing result
Patient 01	Positive	Positive	7	9	24.9	35.7	N/A
Patient 02	Positive	Positive	0	3	18.1	30.0	N/A
Patient 03	Positive	Positive	2	5	19.3	25.0	N/A
Patient 04	Positive	Positive	7	9	33.4	33.5	N/A
Patient 05	Positive	Positive	9	12	33.2	35.0	N/A
Patient 06	Positive	Positive	3	14	23.6	37.1	N/A
Patient 07	Positive	Positive	7	12	22.4	38.7	N/A
Patient 08	Positive	Positive	7	11	28.8	30.0	N/A
Patient 09	Positive	Positive	9	13	28.4	39.2	N/A
Patient 10	Positive	Positive	19	9	17.5	34.7	N/A
Patient 11	Positive	Positive	5	12	18.9	30.5	N/A
Patient 12	Positive	Positive	7	11	31.0	40.0	N/A
Patient 13	Positive	Negative	2	6	21.7	N/A	Indeterminate (day 3)
Patient 14	Positive	Positive	43	29	29.1	26.2	N/A
Patient 15	Positive	Positive	1	13	17.1	36.8	Positive (day 23)
Patient 16	Positive	Positive	1	9	29.1	34.8	N/A
Patient 17	Positive	Positive	0	2	25.1	36.8	N/A

Abbreviations: C_T: cycle threshold, NP: nasopharyngeal, RT-PCR: reverse transcriptase polymerase chain reaction

Supplementary Figure 1

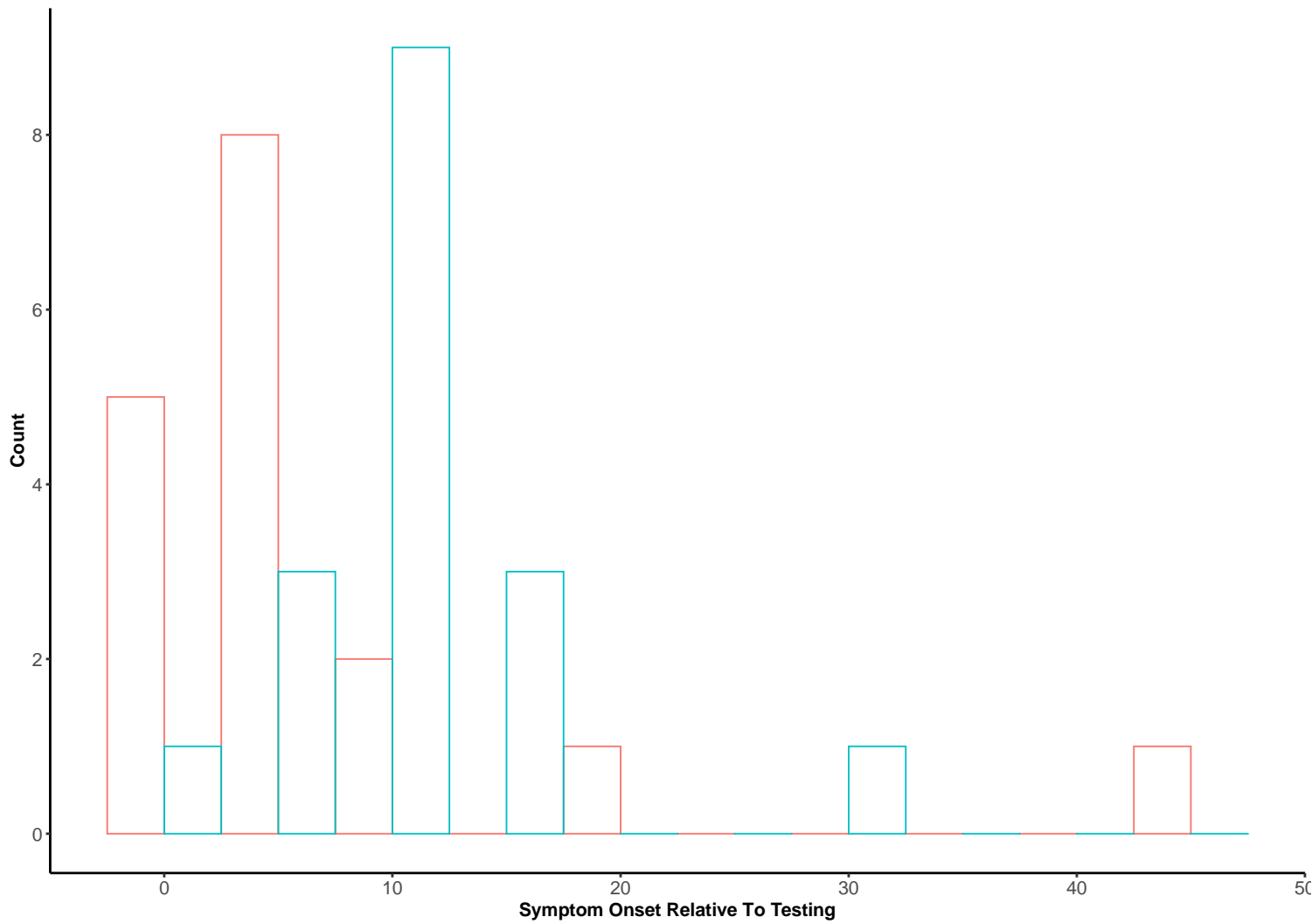


Figure Legend. Histogram of symptom onset relative to nasopharyngeal (red) and stool (blue) testing (n=17). Median [range] days between symptom onset and nasopharyngeal and stool RT-PCR testing was 7 [1-43] days and 11 [3-29] days respectively.

Supplementary Figure 2

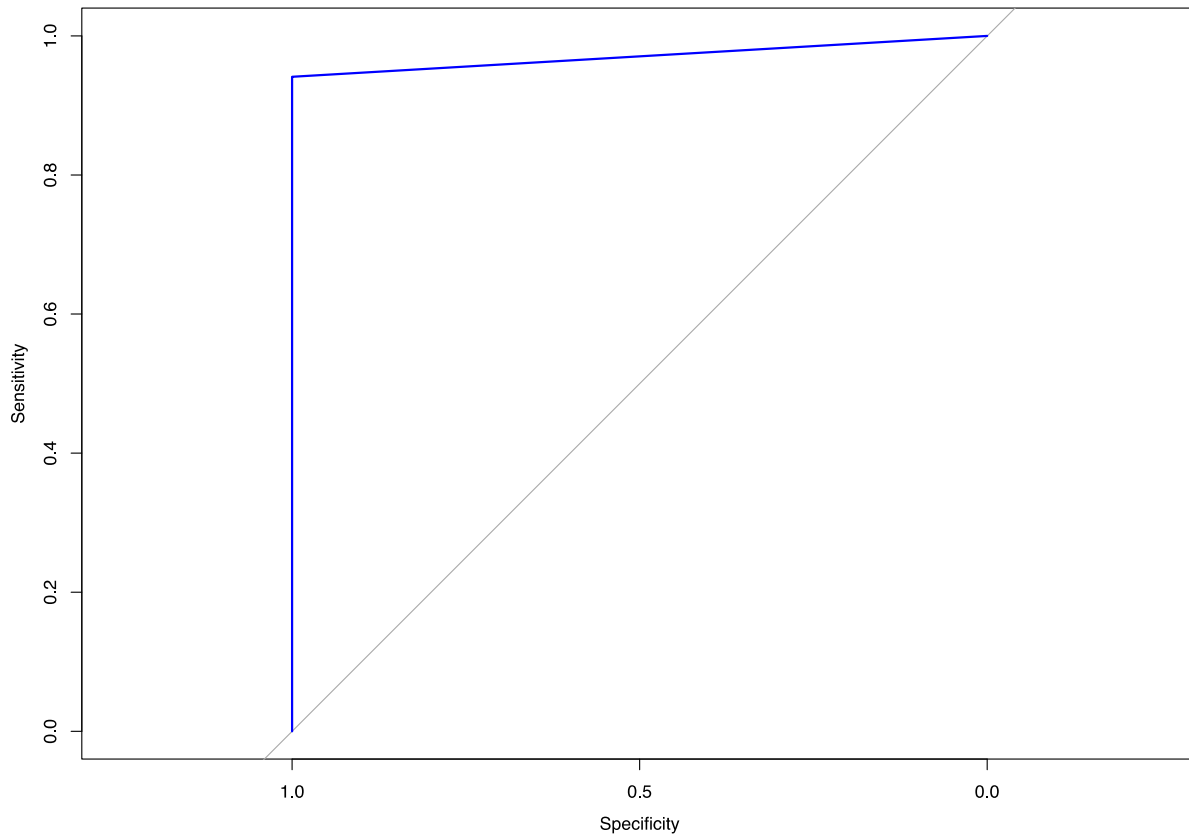


Figure Legend. Receiver Operating Characteristic curve. The performance of stool RT-PCR compared to NP RT-PCR was compared using a receiver operating characteristic curve (ROC) analysis. The area under the ROC curve (AUC) was determined to be 0.971

Supplementary Figure 3

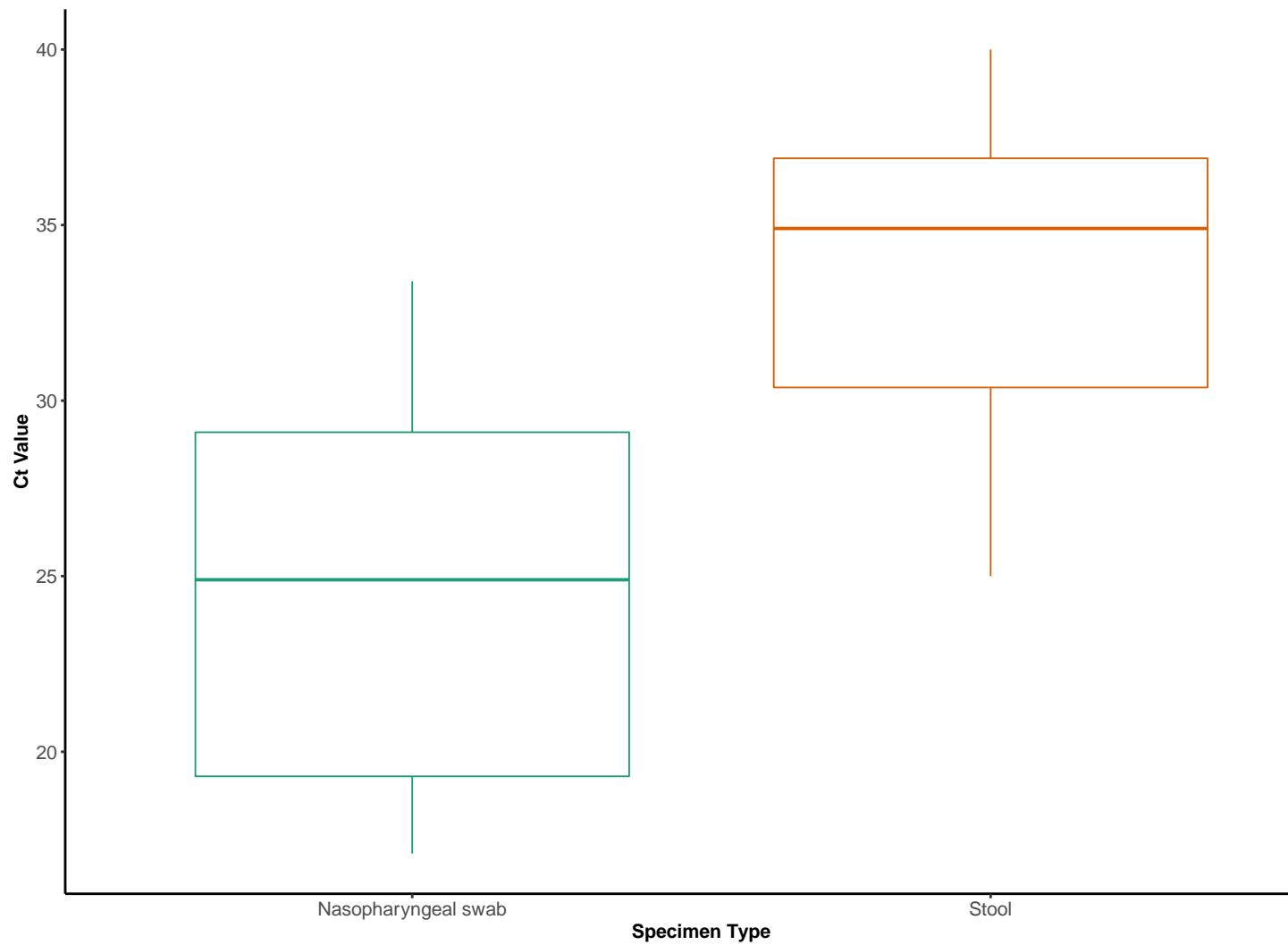


Figure Legend. Box plot of nasopharyngeal swab C_T values (n=17) and stool C_T values (n=16). Median stool C_T value was significantly higher than median nasopharyngeal swab C_T value (34.9 [6.5] vs. 24.9 [9.8]; p value <0.001)

Supplementary Figure 4

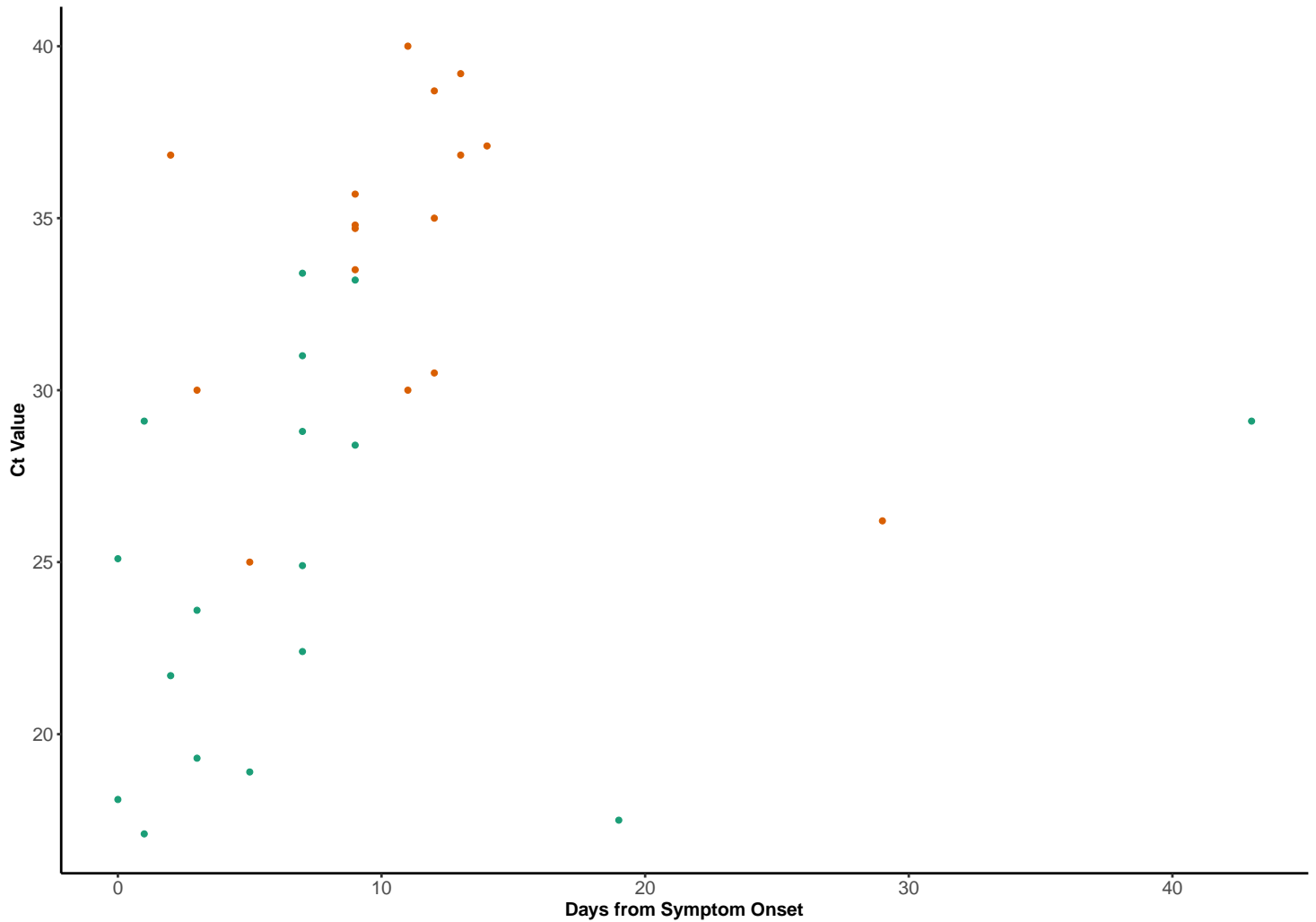


Figure Legend. Scatter plot of nasopharyngeal swab (green) C_T values ($n=17$) and stool (red) C_T values ($n=16$) by day of testing relative to symptom onset. Median stool C_T value was significantly higher than median nasopharyngeal swab C_T value (34.9 [6.5] vs. 24.9 [9.8]; p value <0.001). Median [range] days between symptom onset and nasopharyngeal and stool RT-PCR testing was 7 [1-43] days and 11 [3-29] days respectively.

Supplementary Figure 4

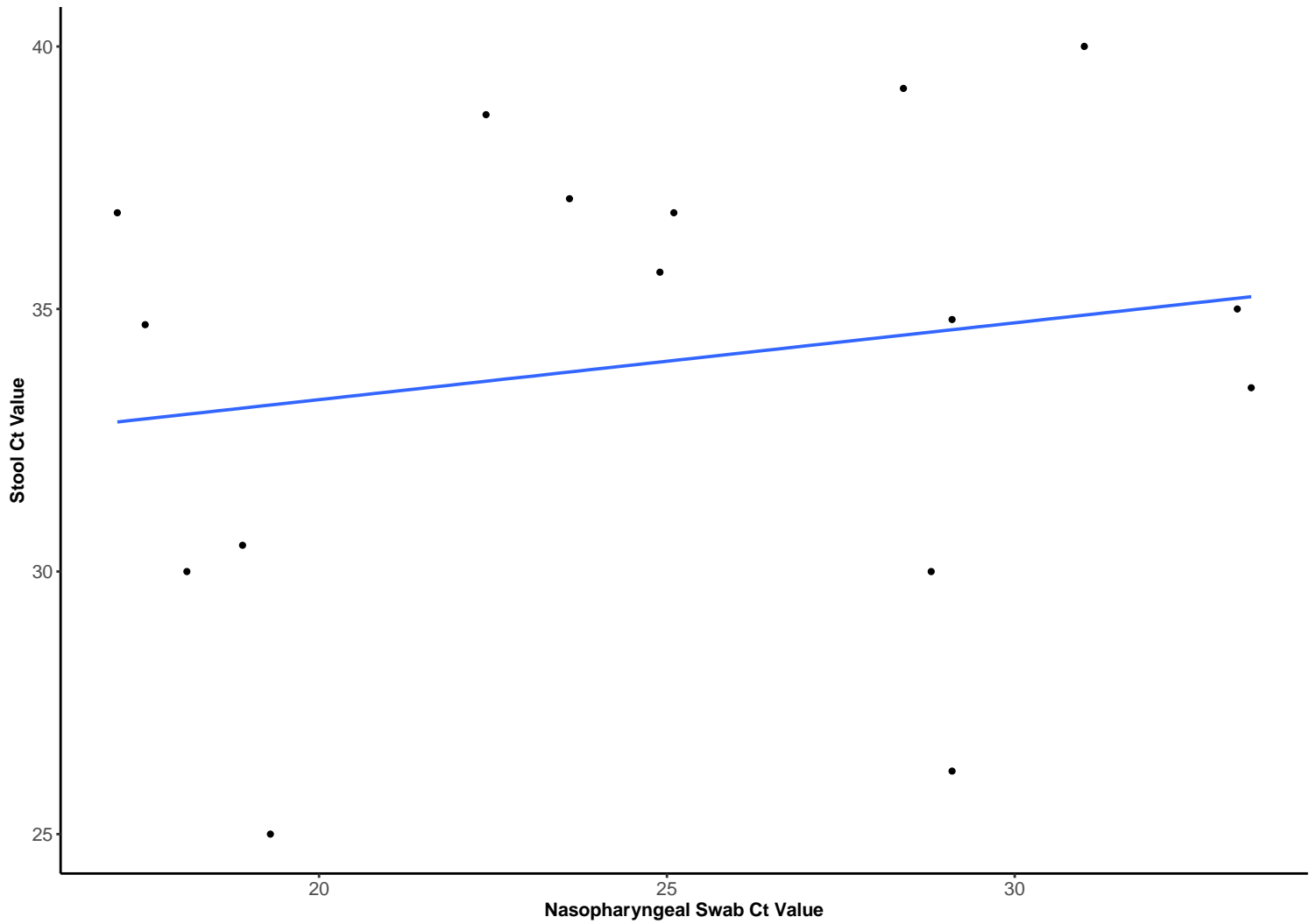


Figure Legend. Correlation of C_T values for stool and nasopharyngeal SARS-CoV-2 RT-PCR assays, (n=16). Nasopharyngeal swab C_T values are plotted versus stool C_T . Stool C_T values did not correlate with NP C_T values (R^2 : -0.04, p value = 0.50)

References

1. Babiker A, Myers CW, Hill CE, et al. SARS-CoV-2 Testing. *Am J Clin Pathol* 2020;153:706-708.
2. Lu X, Wang L, Sakthivel SK, et al. US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2. *Emerg Infect Dis* 2020;26:1654-65.
3. Centers for Disease Control and Prevention. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. CDC/DDID/NCIRD/ Division of Viral Diseases, 2020.