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VIROLOGY

Utility of Stool PCR for the Diagnosis of COVID-19: Comparison of Two Commercial Platforms

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ABSTRACT The ability to detect SARS-CoV-2 in the upper respiratory tract ceases after 2 to 3 weeks post-symptom-onset in most patients. In contrast, SARS-CoV-2 can be detected in the stool of some patients for greater than 4 weeks, suggesting that stool may hold utility as an additional source for diagnosis. We validated the Cepheid Xpert Xpress SARS-CoV-2 and Hologic Panther Fusion real-time RT-PCR assays for detection of viral RNA in stool specimens and compared performance. We utilized remnant stool specimens ($n = 79$) from 77 patients with gastrointestinal symptoms. Forty-eight patients had PCR-confirmed COVID-19, and 29 either were nasopharyngeal/oropharyngeal PCR negative or presented for reasons unrelated to COVID-19 and were not tested. Positive percent agreement between the Cepheid and Hologic assays was 93% (95% confidence interval [CI]: 81.1% to 98.2%), and negative percent agreement was 96% (95% CI: 89% to 0.99%). Four discrepant specimens (Cepheid positive only, $n = 2$; Hologic positive only, $n = 2$) exhibited average cycle threshold (C_T) values of >37 for the targets detected. Of the 48 patients with PCR-confirmed COVID-19, 23 were positive by both assays (47.9%). For the negative patient group, 2/29 were positive by both assays (6.9%). The two stool PCR-positive, nasopharyngeal/oropharyngeal PCR-negative patients were SARS-CoV-2 IgG positive. Our results demonstrate acceptable agreement between two commercially available molecular assays and support the use of stool PCR to confirm diagnosis when SARS-CoV-2 is undetectable in the upper respiratory tract.

KEYWORDS COVID-19, SARS-CoV-2, diagnostics, stool PCR

Diagnostic testing for acute SARS-CoV-2 infection is primarily accomplished by PCR-based assays performed on upper respiratory specimens due to high viral load and the relative ease of specimen collection (1–3). Viral RNA levels peak in the oropharynx and nasopharynx between 4 and 6 days after symptom onset and remain detectable for a median duration of 15 to 18 days (1, 2, 4–7). Bronchial alveolar lavage fluid and sputum have been found to be more frequently positive than pharyngeal swabs (8, 9), but lower respiratory specimens are not routinely tested due to the increased risk of exposure to health care personnel. For patients with negative SARS-CoV-2 respiratory PCR results but symptoms compatible with COVID-19, serologic testing can support diagnosis but cannot be used as the sole basis to diagnose the disease (10). Additionally, antibodies may not be detectable early in the disease course and the specificity of available serology assays is highly variable (11, 12).

Glandular epithelial cells in the stomach, duodenum, and rectum can be infected with SARS-CoV-2 (13). Virus has been cultured from a small number of stool specimens (8, 14), and SARS-CoV-2 RNA is detectable in stools from patients with and without

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gastrointestinal symptoms (5, 15, 16). Between 32 and 67% of PCR-confirmed COVID-19 patients have detectable SARS-CoV-2 RNA in stool, and multiple studies have demonstrated persistence of viral RNA in stool for longer periods than recoverable in the upper respiratory tract (5, 7, 8, 13, 15, 17). Patients with both severe and mild disease have been found to shed viral RNA for >4 weeks after symptom onset, at which time there is no detection in the upper respiratory tract (6, 7, 18). Because of the prolonged shedding observed in some patients, stool PCR may aid in the diagnosis of COVID-19 when upper respiratory specimen testing is negative but clinical suspicion of disease remains high. Cases where stool was the only specimen in which SARS-CoV-2 could be detected have been reported (18, 19). Finally, the extended period for which viral RNA is recoverable in stool may provide distinct value in the diagnostic workup of pediatric patients presenting with symptoms suggestive of multisystem inflammatory syndrome in children (MIS-C) (20–22).

We sought to validate the Cepheid Xpert Xpress SARS-CoV-2 and Hologic Panther Fusion real-time RT-PCR assays for the detection of viral RNA in stool specimens. The Cepheid and Hologic assays have emergency use authorization (EUA) approval for the testing of upper respiratory specimens, and clinical evaluations have demonstrated high levels of agreement between the Cepheid and Hologic assays and with other molecular tests (23–25). We also performed a clinical evaluation of the Cepheid and Hologic platforms using 79 remnant stool specimens from 77 patients, 48 of which had upper respiratory PCR-confirmed COVID-19.

MATERIALS AND METHODS

Stool specimens and patient clinical characteristics. A convenience sample of remnant stool specimens submitted to the Clinical Microbiology Laboratory at Montefiore Medical Center for routine diagnostic testing was utilized. Stool specimens were collected between 21 April and 15 May 2020 and were tested for SARS-CoV-2 within 7 days of collection. Stool specimens were stored at 2 to 8°C prior to initial real-time RT-PCR testing, which was first performed using the Cepheid platform. Chart review of patients from whom stool was utilized was performed to identify all SARS-CoV-2 nasopharyngeal RT-PCR and serology results. Specimens for routine PCR testing during the study period consisted of combined nasopharyngeal/oropharyngeal swabs transported in universal transport medium (BD, Sparks, MD) or ESwab (Copan, Italy) collection devices. Clinical testing of the swab PCR was performed using multiple platforms (Cepheid, Hologic Panther Fusion, Abbott M2000, and Luminex Aries). Additional IgG serologic testing was performed using the Abbott SARS-CoV-2 immunoassay on the Architect i2000sr instrument. In total, 79 stool specimens from 77 unique patients were used for comparison testing. This study was approved by the Albert Einstein College of Medicine IRB (2018-9587).

Stool specimen SARS-CoV-2 real-time RT-PCR testing. Stool was tested by submerging a rayon-tipped swab into several areas of the specimen to obtain a coating of stool, followed by transfer into 1 ml of 0.85% normal saline. Real-time RT-PCR was then performed using the Xpert Xpress SARS-CoV-2 or Hologic Panther Fusion assays following the package insert instructions for upper respiratory specimen testing. For the Cepheid assay, the GeneXpert Infinity instrument running software version 6.8 was utilized. The Cepheid assay detects the E and N2 gene of SARS-CoV-2 and contains a processing control to ensure extraction and amplification (26). The Hologic assay was performed on the Panther Fusion system. The Hologic assay detects two ORF1a regions of SARS-CoV-2 and also contains an internal control but utilizes only one fluorescent channel for reporting of the ORF1a amplification product (27).

Validation of the Cepheid and Hologic SARS-CoV-2 assays for stool specimens. For limit of detection (LoD) studies, a PCR-negative liquid stool specimen was transferred to saline as described above and spiked with SeraCare AccuPlex SARS-CoV-2 reference or verification material (Milford, MA). The SeraCare control material is composed of recombinant alphavirus particles containing E, N2, ORF1a, and RdRp genes. LoD studies were first performed for the Cepheid assay using the SeraCare reference control, which has a concentration of 5,000 copies/ml. The stool-saline mixture was spiked with the SeraCare reference control at concentrations of approximately 2,500, 1,250, and 625 copies/ml. At a later date, the SeraCare verification material was used to determine the LoD of the Hologic assay. Use of the SeraCare verification control (100,000 copies/ml) allowed for a wider range of concentrations to be tested: 50,000, 5,000, 2,500, 1,250, 625, and 312.5 copies/ml. The LoD for each platform was confirmed by performing 20 replicates and achieving at least a 95% detection rate. For calculations of average cycle threshold (C_T) and standard deviation values for the Cepheid assay, nondetected targets were excluded from the analyses.

Cross-reactivity testing was assessed on both the Cepheid and Hologic platforms by testing a panel of 10 bacterial organisms that can be found in the gastrointestinal tract (*Bacteroides fragilis* ATCC 23745, *Clostridium difficile* ATCC 70057, *Prevotella melaninogenica* ATCC 25245, *Clostridium perfringens* ATCC 13124, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 33186, *Escherichia coli* ATCC 13846, *Enterobacter cloacae* ATCC 23355, and *Citrobacter freundii* ATCC 80900). A suspension of each organism in saline was prepared at an initial concentration of >1.0 McFarland standard (1×10^8 to 1.5×10^8 CFU/ml) and then diluted 1:100 in

TABLE 1 Results of limit of detection determination for the Cepheid and Hologic assays

No. of copies/ml SeraCare control material	Assay used for testing	No. of replicates positive/tested	Mean C_T ± SD for gene:		
			E	N2	ORF1a
1,250	Cepheid	19/20	38.1 ± 1.3	40.5 ± 1.1	NA[a]
2,500	Hologic	20/20	NA	NA	36.3 ± 0.88

[a]NA, not available.

saline to obtain a final concentration of $>10^6$ CFU/ml. FilmArray gastrointestinal pathogen panel controls M239 and M240 (Maine Molecular Quality Controls, Inc., Saco, ME) which contain RNA from *Clostridium difficile* toxin A/B, *Plesiomonas shigelloides*, *Vibrio cholerae*, *Yersinia enterocolitica*, enteroaggregative *Escherichia coli*, Shiga-like-toxin-producing *E. coli* O157, *Shigella*/enteroinvasive *E. coli*, *Cryptosporidium*, *Campylobacter*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, astrovirus, norovirus, and rotavirus A were also tested.

To assess accuracy, 30 negative stool specimens were spiked with SeraCare control material at the LoD ($n = 20$) and 2× the LOD ($n = 10$) and tested on the Cepheid platform. An additional 10 specimens spiked at the LoD were tested on the Hologic platform. Interassay precision was determined by having three operators perform the Cepheid assay on three stool specimens and by testing the three specimens over three consecutive days. Specimens were held at 2 to 8°C over the 3-day testing period.

Specimen preparation for comparison studies. Testing on the Cepheid platform was performed using stool specimens stored at 2 to 8°C for 1 to 7 days. Multiple aliquots of the stool specimens were prepared at the time of initial testing by transferring stool as described into 1 ml of 0.85% saline. Some specimens were frozen at −80°C prior to testing on the Hologic Panther Fusion platform. For discrepant specimens, testing was repeated on both platforms using a new aliquot and a shared aliquot tested on both instruments on the same day.

Statistical analyses. Assay comparison data were used to construct 2 × 2 tables for positive percent and negative percent agreement and 95% confidence interval calculations. Cohen's kappa coefficient was used to assess the agreement between the Cepheid and Hologic platforms. A value of <0.4 was considered low agreement; 0.4 to 0.6, moderate agreement; 0.61 to 0.8 substantial agreement; and 0.81 to 1.0, near or perfect agreement. Fisher's exact test was performed to compare assay agreement, and Wilcoxon matched-pairs signed-rank 2-tailed test was used to compare E and N2 values. *P* values of ≤0.05 were considered statistically significant. Prism version 7 was used for statistical analyses.

RESULTS

Analytical performance. We developed a protocol to use the Cepheid Xpert Express and Hologic Panther Fusion SARS-CoV-2 real-time PCR assays for the testing of stool specimens. The amount of stool used to perform the testing mirrors precisely the protocols described for the Cepheid *Clostridium difficile*/Epi and CarbaR assays, in which the swab is placed into the stool but not completely saturated (28, 29).

To determine the LoD of the Cepheid and Hologic assays, we tested dilutions of SeraCare SARS-CoV-2 control material in the liquid stool-saline matrix. For the Cepheid assay, the E or N2 genes were detected in 19/20 (95%) replicates spiked at 1,250 copies/ml. The Hologic assay LoD was established as 2,500 copies/ml, with all samples (20/20) testing positive (Table 1).

To ensure that cross-reactivity with stool organism did not occur, we tested a panel of 10 gastrointestinal bacteria (*B. fragilis*, *C. difficile*, *P. melaninogenica*, *C. perfringens*, *P. mirabilis*, *K. pneumoniae*, *E. faecalis*, *E. coli*, *E. cloacae*, and *C. freundii*) and control material containing RNA from 17 different gastrointestinal pathogens. We obtained negative results for all organisms and control material tested on both the Cepheid and Hologic platforms.

Accuracy was assessed by spiking negative stool specimens from unique patients with SeraCare control material. For the Cepheid assay, we detected the E or N2 gene in 19/20 (95%) samples spiked at the LoD and 10/10 (100%) samples at 2× the LoD. An additional 10 specimens were spiked at the LoD of the Hologic assay, and SARS-CoV-2 was detected in 10/10 (100%).

Studies to determine interassay precision were performed on the Cepheid platform. Three medical technologists performed Cepheid testing on three positive clinical specimens and obtained positive results for all specimens tested (9/9, 100%). The C_T values (mean ± standard deviation) for the E and N2 genes were as follows: 29.2 ± 1.14, 32.8 ± 1.76, and 34.8 ± 2.48, and 36.2 ± 0.81, 35.2 ± 0.58, and 39.5 ± 1.48, respectively.

TABLE 2 Result agreement between Cepheid and Hologic Panther Fusion assays ($n = 79$)

	Hologic Panther Fusion SARS-CoV-2 assay		% agreement (95% CI) between assays	
Cepheid SARS-CoV-2 assay	Pos (*n*)	Neg (*n*)	Pos	Neg
Pos (*n*)	27	2	93 (81.1–98.2)	96 (89.0–99.0)
Neg (*n*)	2	48		

We also tested the three clinical specimens over three different days and obtained positive results for all specimens (9/9, 100%).

Clinical evaluation. We utilized remnant clinical stool specimens ($n = 79$) from patients with symptomatic diarrhea to compare the Cepheid and Hologic Panther Fusion assays. We obtained positive and negative agreement for 27 and 48 stool specimens, respectively. There were four discrepancies. The positive percent agreement between the assays was 93% (95% confidence interval [CI]: 81.1% to 98.2%), and negative percent agreement was 96% (95% CI: 0.89% to 0.99%) (Table 2). Cohen's kappa for agreement was 0.867 (95% CI: 74.0% to 99.3%) indicating near-perfect agreement ($P < 0.0001$).

For specimens testing positive by both the Cepheid and Hologic assays, we observed a wide range of C_T values for the E (19.1 to 41.3), N2 (21.6 to 41.3) and ORF1a (20.1 to 38.0) targets. Average and standard deviation for each target were 31.9 ± 6.0 for E, 35.1 ± 6.1 for N2, and 32.6 ± 5.6 for ORF1a. For the four discrepant stool specimens, two were positive only by the Cepheid assay and two were positive only by Hologic. The discrepant specimens were repeated two times on the Cepheid platform and once on Hologic, and all repeat results were consistent with the original results. Average C_T values of replicates for discrepant specimens were >37. All four discrepant specimens originated from patients with PCR-confirmed disease or positive IgG serology results (Table 3).

For the stool specimens testing positive by both assays ($n = 27$), we saw good correlation (R^2) between the Hologic ORF1a and Cepheid C_T values for E (0.8382) and N2 (0.8616) targets (Fig. 1). We did not find correlation between C_T values and time since first nasopharyngeal/oropharyngeal PCR results (E, 0.0046; N2, 0.00003) (Fig. 2). C_T values of the Cepheid gene targets for the positive stool specimens were examined, and E gene amplification (average $C_T \pm$ standard deviation) was found to occur earlier (33.0 ± 5.28) than the N2 gene (35.9 ± 4.9) ($P < 0.0001$). The E gene was detected in all specimens, but the N2 gene was not detected in two specimens.

TABLE 3 SARS-CoV-2 diagnostic testing results for patients with stool PCR results discrepant by the Cepheid and Hologic assays

Patient specimen no.	Nasopharyngeal/ oropharyngeal swab SARS-CoV-2 PCR results[a]	SARS-CoV-2 antibody results (IgG)[b]	Stool collection date	Mean $C_T \pm$ SD for assay and gene		
				Cepheid PCR		Hologic PCR ORF1a
				E	N2	
27	Negative Day 0 Negative Day 1 Negative Day 7	Positive Day 9	Day 17	41.5 ± 1.5	42.7 ± 1.3	Not detected
32	Positive Day 0 Positive Day 43	Positive Day 18	Day 28	37.6 ± 0.9	37.8 ± 2.0	Not detected
46	Positive Day 0 Negative Day 2	Not tested	Day 8	Not detected	Not detected	38.0 ± 0.1
48	Negative Day 0 Negative Day 1 Positive Day 7 Negative Day 13	Not tested	Day 5	Not detected	Not detected	38.2 ± 0.2

[a]Result and day of initial (day 0) and subsequent upper respiratory PCR results.
[b]Result and day of IgG serology results in relation to initial upper respiratory PCR result.

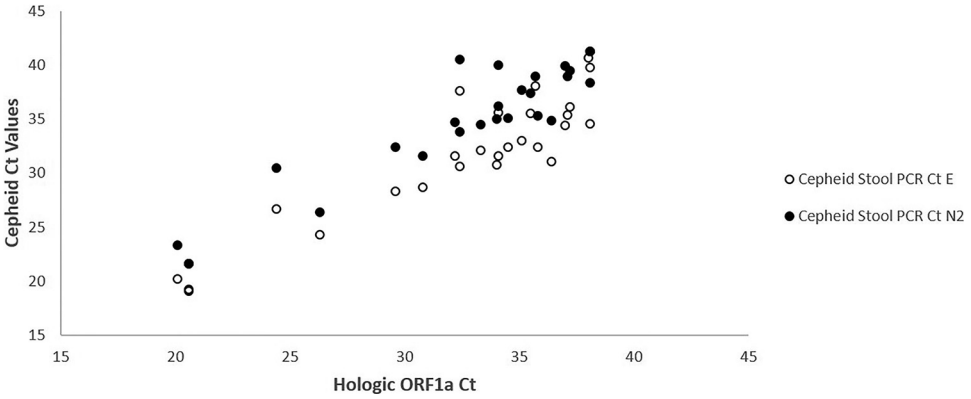

FIG 1 Correlation of C_T values for Cepheid and Hologic Panther Fusion SARS-CoV-2 assays. Hologic ORF1a C_T values are plotted verse Cepheid E and N2 C_T values for stool specimens testing positive by both assays ($n = 27$).

Stool specimens positive for SARS-CoV-2 were collected 0 to 33 days from the time of initial upper respiratory PCR testing (Fig. 2). Eight of the 27 positive stool specimens (29.6%) were collected 14 days or more after the initial diagnostic nasopharyngeal/oropharyngeal specimen was tested (Fig. 2). Six stool specimens were collected at day 21 or later (22.2%).

The stool specimens used for the correlation studies were from patients with positive ($n = 48$), or negative ($n = 23$), nasopharyngeal/oropharyngeal SARS-CoV-2 PCR results and from patients not clinically suspected of COVID-19 and not tested ($n = 6$). Of those with PCR-confirmed disease, 23 of 48 specimens were positive by both assays (47.9%). Two patients had two positive stool specimens from different collections, one of whom had stool specimens collected 11 and 21 days after the initial COVID-19 diagnosis. All six patients not previously tested for SARS-CoV-2 were negative by both assays. Of the 23 patients testing negative by nasopharyngeal/oropharyngeal PCR, we detected SARS-CoV-2 in two stool specimens by both Cepheid and Hologic assays. One of the two patients repeatedly tested negative by nasopharyngeal/oropharyngeal PCR, and ultimately both patients had detectable SARS-CoV-2 IgG (Table 4).

DISCUSSION

PCR testing of upper respiratory specimens is recommended for diagnosis of acute SARS-CoV-2 infection (30), but false-negative results can occur for a variety of reasons, including poor specimen collection, recovery of virus at levels below the LoD of the assay, and biological reasons such as absent or intermittent shedding (31). In this study, we validated the Cepheid Xpert Xpress SARS-CoV-2 and Hologic Panther Fusion real-

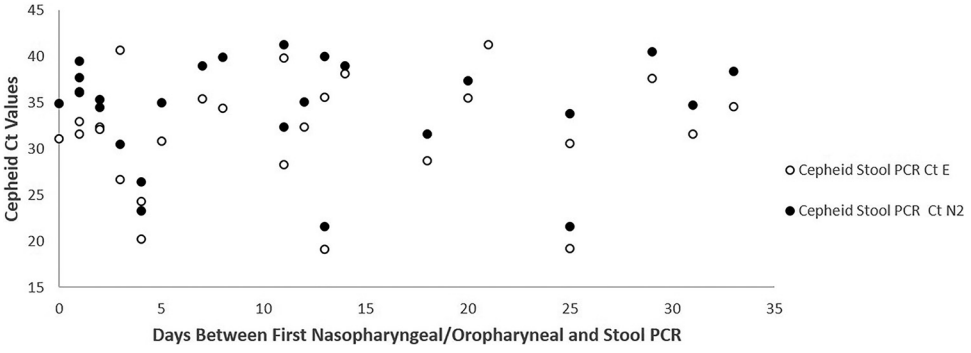

FIG 2 Detection of SARS-CoV-2 assay amplification targets in positive stool specimens up to 33 days after initial upper respiratory PCR result. Cepheid SARS-CoV-2 gene targets for individual patient stool specimens are plotted versus the number of days from the first positive nasopharyngeal/oropharyngeal PCR result ($n = 25$) or first initial negative nasopharyngeal/oropharyngeal PCR for patients with only negative upper respiratory PCR findings ($n = 2$).

TABLE 4 SARS-CoV-2 diagnostic testing results for patients with negative nasopharyngeal/oropharyngeal and positive SARS-CoV-2 PCR results

Patient specimen no.	Nasopharyngeal/oropharyngeal swab SARS-CoV-2 PCR results[a]	SARS-CoV-2 antibody results (IgG)[b]	Stool collection date	C_T for assay and gene		
				Cepheid PCR		
				E	N2	Hologic PCR ORF1a
74	Negative Day 0 Negative Day 3 Negative Day 8 Negative Day 13 Negative Day 22	Positive Day 12	Day 8	34.4	39.9	35.7
20	Negative Day 0 Negative Day 15 (autopsy)	Positive Day 6	Day 6	38.1	39.0	37.0

[a]Result and day of initial (day 0) and subsequent upper respiratory PCR results.
[b]Result and day of IgG serology results in relation to initial upper respiratory PCR result.

time RT-PCR assays for detection of viral RNA in stool specimens to expand diagnostic capability. We were able to detect viral RNA in stool as late as 33 days after initial presentation and in 47.9% of patients with nasopharyngeal/oropharyngeal PCR-confirmed disease, which is consistent with previous studies (5, 7, 8, 13, 17). Furthermore, stool PCR confirmed diagnosis of SARS-CoV-2 for two patients who were negative by upper respiratory tract PCR testing.

We found the Cepheid and Hologic assays accurate for the detection of SARS-CoV-2 in stool specimens. The two assays exhibited comparable limits of detection with the Cepheid assay only 1 dilution lower than that of Hologic. However, the LoD for stool testing (1,250 to 2,500 copies/ml) is higher than that for nasopharyngeal swab specimens. The Cepheid assay has a manufacturer claim of 250 copies/ml for nasopharyngeal swab specimens (26), and one study demonstrated an even lower LoD of 100 copies/ml (25). The Hologic assay has a claimed LoD of 0.01 50% tissue culture infective dose ($TCID_{50}$)/ml (27), and an LoD of 62.5 copies/ml or 1,000 copies/ml was obtained using synthetic RNA reference material or whole inactivated virus, respectively (32). The higher LoD for stool than upper respiratory specimens may be due to the presence of PCR inhibitors (33). No cross-reactivity was observed for either assay, and we were able to detect viral RNA in 95% (Cepheid) and 100% (Hologic) of clinical stool specimens spiked with SARS-CoV-2 RNA at the limit of detection, demonstrating the robustness of the assays across multiple stool matrices. Stool testing was precise, despite the use of a swab-based transfer protocol that relies on the subjective observation of a stool coating instead of a measurable amount of specimen. We found 100% concordance between replicate samples from testing performed by different technologists. Also, we did not have any test failures related to failed amplification of the internal control regardless of the numerous PCR inhibitors that can be present in stool (33). It should be noted that some samples required repeat processing on the Hologic platform because of robotic pipetting errors that may have arisen from high sample viscosity or fecal material clumping. For the Cepheid assay, the samples are manually added to the testing cartridge using a transfer pipette, alleviating the potential for automated processing errors.

Clinical evaluation of the Cepheid and Hologic assays revealed excellent positive percent agreement (93%) and negative percent agreement (96%), and we saw good correlation between Hologic and Cepheid C_T values. The discrepancies observed in the clinical evaluation occurred for specimens with high C_T values of >37, suggesting low viral load. The Cepheid and Hologic assays detect different gene targets, which may account for the discordance. Target amplification differences for the E and N2 genes were found in our study and have been previously reported for upper respiratory specimens, where the E gene was similarly found to exhibit lower C_T values than the N2 target (23).

The addition of PCR testing of stool confirmed disease for two patients in our study: one patient with repeated nasopharyngeal/oropharyngeal negative PCR results and one patient who expired after developing respiratory failure. For the patient who expired, nasopharyngeal/oropharyngeal swab PCR results were negative at time of initial presentation and at autopsy. The ability to detect virus at the time of death may obviate a full autopsy for those with PCR-confirmed disease, which may be necessary for facilities with limited autopsy capacity.

Stool PCR testing may hold value for patients presenting late in the disease course who are more likely to have negative upper respiratory PCR results (4, 5, 7). For example, children with MIS-C present weeks after initial infection, and most do not have detectable virus in the upper respiratory tract. In the first case series describing the syndrome, none of the children had positive PCR results at presentation, although two tested positive for SARS-CoV-2 at later dates (21). Similarly, in two series from Europe only 20 to 34% of children tested positive by upper respiratory PCR (20, 22). Positive stool PCR results were reported for two children with MIS-C (20), suggesting a potential value for stool testing. Over 80% of children with MIS-C have a detectable SARS-CoV-2 antibody response (20, 22), and most patients develop IgG within 19 days of illness onset (11); therefore, serologic testing may support the diagnosis for those presenting late in the disease course. However, PCR-based assays offer the assurance of high specificity in contrast to serologic assays that have the potential to cross-react with seasonal coronaviruses and for which limited performance data are available (10, 34). In our study we were able to detect SARS-CoV-2 in stool collected greater than 4 weeks after initial presentation, providing further support for stool PCR testing for patients presenting weeks after initial infection.

The ability to detect viral RNA in stool specimens and wastewater has raised concerns for fecal transmission (35, 36), but no studies to date have demonstrated infection via a fecal matter-associated route. A limited number of studies have sought to determine if live virus can be recovered from stool specimens. In one report, viral isolation was attempted from four stool specimens that had high copy numbers of viral RNA suggestive of high viral loads. The authors state that they were able to culture and observe virus from two specimens using electron microscopy, but details of the methodology were not provided (8). More recently, virus isolation from stool was again reported. Using Vero E cells, the authors observed cytopathic effect 2 days after second-round passage, and they were able to obtain full-length viral genome sequence from culture supernatant in addition to confirming the presence of viral particles using transmission electron microscopy (14). In contrast, virus was not recoverable from 10 stool specimens in another study and *in vitro* experiments using a simulated human colonic fluid demonstrated SARS-CoV-2 inactivation, suggesting that fecal transmission is unlikely (37). Our study did not aim to answer any infection control questions, and we do not advise that stool testing be implemented for screening purposes outside fecal donation, which is recommended by the FDA (38). It is important to recognize that some patients with COVID-19 will present with gastrointestinal symptoms (39), and rapid diagnosis is needed for patient management. Future studies that address the sensitivity of stool verse upper respiratory PCR testing for patients presenting with gastrointestinal symptoms may help to refine diagnostic algorithms.

Limitations of our study include the use of a convenience collection of remnant stool specimens stored for varying times between 1 and 7 days at 2 to 8°C prior to testing. Also, we did not use an arbitrator method to resolve discrepancies. Our findings that 47.9% of nasopharyngeal/oropharyngeal PCR-confirmed patients had detectable viral RNA in stool are in alignment with previous findings (5, 7, 8, 13, 17), but our study was not designed to determine the prevalence of stool positivity.

In summary, we found that the Cepheid Xpert Xpress SARS-CoV-2 and Hologic Panther Fusion assays can be used for the reliable detection of viral RNA in stool specimens, and our results suggest that inclusion of stool PCR in the testing algorithm may increase clinical sensitivity. The ability to confirm diagnosis in those with negative upper respiratory PCR results may be important for establishing the initial diagnosis

and expanding access to clinical trials that require PCR positivity (40). However, stool testing cannot solely be used to rule out disease since not all patients shed viral RNA in stool. We caution that the use of stool testing should be limited and utilized only after results of upper respiratory PCR are available. Reagent shortages emphasize a need to perform additional testing of any nature, including repeat testing of nasopharyngeal specimens, only when clinically appropriate.

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