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RESEARCH ARTICLE

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The Hologic Aptima SARS-CoV-2 assay enables high ratio pooling saving reagents and improving turnaround time

Kimberly Newsom | **Yuan Zhang** | **Srikar Chamala** | **Katherine Martinez** | **Michael Clare-Salzler** | **Petr Starostik**

Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, USA

Correspondence
Petr Starostik, Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, USA.
Email: starostik@pathology.ufl.edu

Abstract

Background: The Hologic Aptima™ TMA SARS-CoV-2 assay was employed to test pooled nasopharyngeal (NP) samples to evaluate the performance of pooled sample testing and characterize variables influencing results.

Methods: Results on 1033 previously tested NP samples were retrieved to characterize the relative light units (RLU) of SARS-CoV-2-positive samples in the tested population. The pooling strategy of combining 10 SARS-CoV-2 samples into one pool (10/1) was used in this study. The results were compared with neat sample testing using the same Aptima™ TMA SARS-CoV-2 assay and also the CDC RT-PCR and the Cepheid SARS-CoV-2 assays.

Results: The Aptima assay compares favorably with both CDC RT-PCR and the Cepheid SARS-CoV-2 assays. Once samples are pooled 10 to 1 as in our experiments, the resulting signal strength of the assay suffers. A divide opens between pools assembled from strong-positive versus only weak-positive samples. Pools of the former can be reliably detected with positive percent agreement (PPA) of 95.2%, while pools of the latter are frequently misclassified as negative with PPA of 40%. When the weak-positive samples with kRLU value lower than 1012 constitute 3.4% of the total sample profile, the assay PPA approaches 93.4% suggesting that 10/1 pooled sample testing by the Aptima assay is an effective screening tool for SARS-CoV-2.

Conclusion: Performing pooled testing, one should monitor the weak positives with kRLU lower than 1012 or quantification cycle (Cq) value higher than 35 on an ongoing basis and adjust pooling approaches to avoid reporting false negatives.

KEYWORDS
pooling samples, SARS-CoV-2, screening test, transcription-mediated amplification

1 | INTRODUCTION

Until the end of September 2020, the rapid spread of severe acute respiratory syndrome coronavirus (SARS-CoV-2) has led to about 34 million cases of coronavirus disease 2019 (COVID-19) worldwide causing almost 1 million deaths.[1] Identification of infected individuals through mass screening tests for SARS-CoV-2 is a crucial way to prevent the spread of the disease. As schools and business reopen or are

Newsom and Zhang equally contributed to this study.

trying to stay opened, expanding testing capacity through accurate, easy to use, and high-throughput molecular diagnostic methods becomes urgent.

So far, more than 160 molecular diagnostic assays for the detection of SARS-CoV-2 have been approved by the Food and Drug Administration (FDA) under the Emergency Use Authorization (EUA). In May 2020, the Hologic® Aptima SARS-CoV-2 assay based on transcription-mediated amplification (TMA) received FDA approval (EUA200734) (Aptima™, Hologic® Panther System). TMA is an isothermal, auto-catalytic target amplification method. The assay shows high sensitivity for SARS-CoV-2 detection and high correlation with the CDC RT-PCR assay.[2,3] Most importantly, the Hologic® Panther platform is a complete sample-to-result automated instrument for testing up to 1200 samples/day/instrument. It thus enables high-throughput testing with minimum manual labor involvement.

With the ever-increasing demand for SARS-CoV-2 testing capacities, pooling of samples for testing becomes a viable alternative in an environment strapped for resources, reagents, and consumables. Sample pooling implies that several samples are mixed together at a given ratio and tested as one single pool. Only positive pools have to be deconvoluted and individual neat samples tested. Otherwise, negative pools are assumed to contain negative samples. The practice of pooling biological samples together for testing is not a new technique, it can be traced back to at least 1940s[4] when it was used for syphilis outbreak testing. It is suggested that testing of pooled samples is better to use for surveillance samples from populations with low prevalence of an infectious agent.[5]

Different sample pooling methods were validated to be used in SARS-CoV-2 testing by many laboratories.[6–10] However, all the previously validated sample pooling methods used the RT-PCR assay. Recently, testing pooled samples with the Hologic Aptima™ TMA assay has been approved by the FDA (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-molecular). In this study, we evaluated the performance of the Aptima™ TMA assay from Hologic® pooling 10 samples together. We explored pooling in a screening setting, testing asymptomatic individuals returning to work or school when the prevalence of positive cases in the population tested was 0.3%–0.7%.

2 | MATERIALS AND METHODS

2.1 | Specimen collection

Total of over 50,000 nasopharyngeal (NP) swabs in viral transportation medium (VTM) were processed for clinical SARS-CoV-2 testing at University of Florida (UF) Pathology Laboratories from both symptomatic and asymptomatic subjects between May and September 2020. Residual specimens of these samples which were stored in −80°C were used to validate the described pooling experiments using the Aptima assay.

2.2 | Aptima TMA SARS-CoV-2 assay

This FDA-approved TMA-based assay detects two different conserved regions within the ORF1ab section of the SARS-CoV-2 viral genome using the Hologic® Panther platform. The testing was performed following manufacturer's (Hologic Inc.) instructions. Cut-off value for positive results was set at 580 kRLU for neat samples and 324 for pooled samples.

2.3 | CDC SARS-CoV-2 RT-PCR assay

The CDC 2019-Novel Coronavirus (2019-nCoV) reverse transcription polymerase chain reaction (RT-PCR) Diagnostic Panel, as described by the CDC follows the FDA-approved protocol. N1 and N2 regions of the virus nucleocapsid (N) gene and human RNase P gene (RP) are analyzed in this panel. RNA isolated and purified from the samples using QIAcube HT (QIAGEN) was reverse transcribed to cDNA and subsequently amplified on the QuantStudio 12K (Thermo) real-time PCR Instrument.

2.4 | Cepheid SARS-CoV-2 assay

FDA-approved Xpert Xpress SARS-CoV-2 assay was run on the GeneXpert instrument (Cepheid). The test incorporates sample preparation, nucleic acid extraction, amplification, and detection of viral nucleic acid targets N2 and E.

2.5 | Sample pooling

Hamilton Microlab next-generation sequencing (NGS) STAR liquid handling system (Hamilton Co.) was used to create sample pools using a proprietary program. Briefly, unscrewed collection tubes containing samples were placed into racks that were pulled onto the robot deck, barcodes on the tubes were read and 50 µl of VTM from each of the 10 sequential samples was pipetted into a barcoded Hologic lysis tube. The pooled samples were then loaded onto the Hologic Panther platform for the Aptima assay. Informatics part of the pooling process was described previously.[11]

2.6 | Statistical methods

Differences in quantitative results were evaluated by Student's *t* test. The false-negative rate (FNR), positive percent agreement (PPA) and negative percent agreement (NPA) were calculated using Microsoft Office Excel software. Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software Inc). $p < 0.05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Performance of the Aptima SARS-CoV-2 assay in single sample testing

While the Aptima SARS-CoV2 assay is semiquantitative and the results are reported qualitatively (virus detected or not detected in the EMR), it still delivers a numeric result on the instrument that corresponds to the kilo relative light units (kRLU) detected. To characterize the Aptima SARS-CoV-2-positive samples in the tested population mix, kRLU results on 1033 previously tested NP samples (499 negative and 534 positive) were retrieved. Among the 499 negative samples, the maximum kRLU value was 368 and the minimum kRLU value was 272, with the mean of 293.5 and SD of 8.93 (293.5 ± 8.93). The 534 positive samples showed maximum kRLU value of 1281, minimum value was 616, mean kRLU was 1150.6 with SD of 70.1 (1150.6 ± 70.1). The positive and negative results were completely separated without any overlap (Figure 1A). Of the 534 Aptima-positive samples, only 19 samples (approximating 3.4% of positive results) had kRLUs below the normal range (mean ± 2SD, Figure 1B), indicating low kRLU variation in the distribution of SARS-CoV-2-positive samples. Importantly, the kRLUs of SARS-CoV-2-positive samples were significantly higher than those of the negative samples ($p < 0.0001$). All kRLU values of 534 positive samples were higher than 580, the predefined positive threshold value for neat samples.

3.2 | Determination of positive cutoff for pooled samples

To assess the effect of pooling on the Aptima assay performance, 34 SARS-CoV-2-positive samples from the analyzed population were combined with negative previously tested samples to generate 10 to 1 (10/1) pools. To assure that the whole positive range from strong positive to weak positive was covered, neat positive samples were also analyzed with the CDC RT-PCR assay that generates quantification cycle (Cq) values corresponding to the number of viral copies in the sample in linear fashion (Table 1). Out of 34 samples, with the Aptima assay, the maximum kRLU value was 1212 and the minimum kRLU value was 787, with the mean kRLU value of 1142 and SD of 75.3 (1142 ± 75.3), which were comparable with the positive sample population of 534 samples (1150.6 ± 70.1) described previously (Figure 1). All kRLU values of the 34 positive neat samples were higher than 580, further confirming that the Aptima TMA assay and CDC RT-PCR assay exhibited PPA of 100%, with no false-negative result (FNR = 0). Then, we evaluated the effect of 10/1 pooling on the kRLU values in the 34 positive samples; kRLUs of pooled samples were compared with the values obtained from the corresponding neat samples (Table 1). Mean kRLU value of 34 samples was 1023.5, with SD of 258.2 (1023.5 ± 258.2), indicating a relatively higher variation of kRLU distribution in pooled samples than in neat ones (Figure 2). The lowest kRLU value of 34 pooled samples was 330, therefore, we moved the kRLU cutoff for positives from 580 to 324

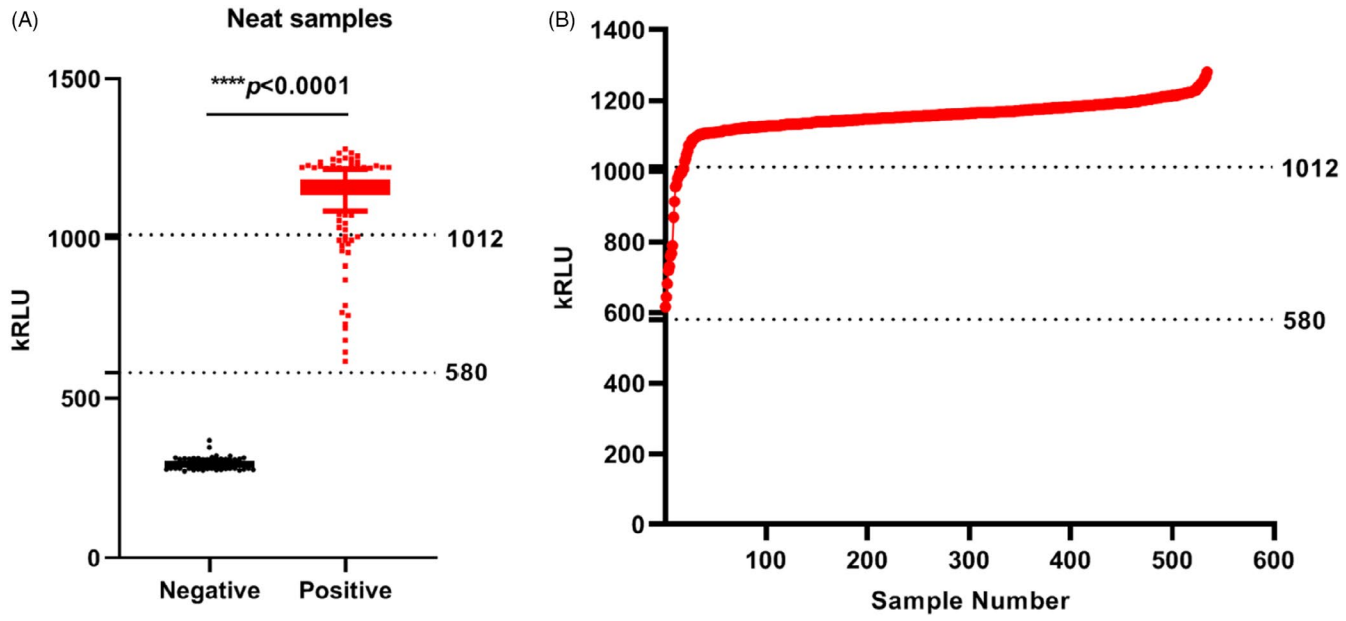


FIGURE 1 Characterization of numeric kRLU results from the Aptima SARS-CoV-2 assay for negative and positive samples. (A) Box plots of 499 previously tested negative and 534 positive NP samples. For the 499 negative samples, the mean kRLU value was 293.5, with SD of 8.93. For the 534 positive samples, the mean kRLU value was1150.6, with SD of 70.1. Data were expressed as mean ± SD. ****$p < 0.0001$ (positives samples vs. negative samples). (B) The distribution of kRLU results in 534 positive samples

TABLE 1 34 SARS-CoV-2-positive neat samples and their 10/1 pools tested with the CDC RT-PCR SARS-CoV-2 and Aptima SARS-CoV-2 Assays

	CDC RT-PCR SARS-CoV-2 Assay (Cq)	Aptima SARS-CoV-2 Assay (kRLU)	
Sample (#)	2019nCoV_N2 Target	NEAT	Pool of 10
1	15.19	1165	1156
2	15.38	1141	1171
3	16.5	1178	1161
4	16.85	1089	1163
5	16.93	1162	1168
6	17.76	1160	1188
7	17.92	1189	1151
8	18.2	1203	1170
9	18.59	1148	1139
10	18.77	1202	1186
11	18.8	1203	1165
12	19.01	1157	1158
13	19.32	1152	1217
14	19.94	1187	1233
15	20.32	1212	1188
16	22.28	1187	1180
17	28.04	1184	1130
18	28.44	1152	1153
19	29.41	1169	1144
20	29.53	1181	1174
21	30.37	1149	1170
22	30.46	1117	1145
23	31.16	1176	1177
24	31.34	1147	365
25	31.97	1167	1033
26	32.25	1150	1061
27	32.25	1129	719
28	33.19	1149	1088
29	33.99	1028	917
30	34.66	1133	498
31	35.74	1131	654
32	36.11	1005	699
33	36.67	1140	549
34	37.92	787	330

to avoid false-negative results (but taking into account increased possibility of false positives that would be discovered and corrected in subsequent neat sample testing). As can be seen in Figure 3A, for both neat samples and their corresponding pools, kRLUs were comparable if N2 Cq of neat samples showed strong signal (Cq <30, samples 1–20). However, neat samples with lower kRLU values showed significantly decreased signal when pooled (Figure 3B). Moreover, the variability of signal detected increased significantly.

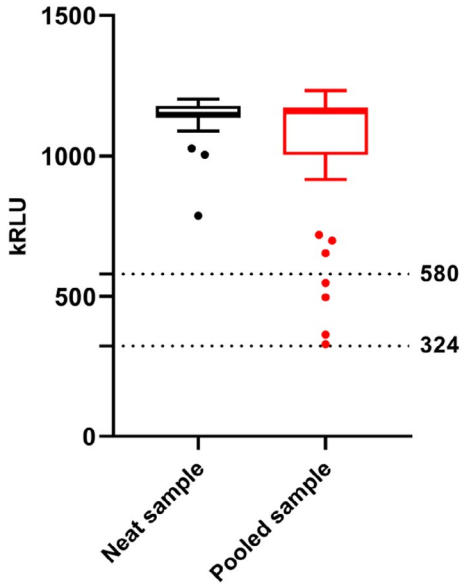


FIGURE 2 Comparison of positive result distribution when tested in neat and pooled formats using the Aptima assay. 34 SARS-CoV-2-positive samples from the analyzed population were tested individually (neat, black) or in 10/1 pools (red). 324 kRLU was established as positivity cutoff for pooled samples

3.3 | Weak-positive pools generate variable signal

To better validate the performance of positive 10/1 pools with a weak signal, additional 23 positive samples from previous Aptima testing were employed. Before the pools were prepared, the neat samples underwent testing with the Cepheid RT-PCR assay to better determine viral copy numbers. Immediately afterwards, these same samples were pooled, run with the Aptima assay in triplicate, and the results plotted (Figure 4C). We decided to run triplicates, as previous testing (Figure 3) showed increased variability of results for weakly positive pooled samples. For neat samples with kRLU results higher than 1012 (Figure 4B), the corresponding pools with one exception (sample #3 that showed also Cepheid assay N2 Cq >37) tested positive, with kRLUs >1000, far higher than the predefined cut-off value of 324 for pooled samples. Moreover, the triplicates showed fairly low variability. Out of 13 weak-positive samples, only 3 pooled samples were tested consistently positive (#11, 13 and 15). In the case of neat samples with kRLU results lower than 1012 (#11-23), the overall performance of their corresponding pools was much worse. We ran the neat samples with the Cepheid RT-PCR assay right before pooling in order to better assess the viral load in the samples and to make sure the viral RNA did not degrade during storage, as the original Aptima results for neat samples were several weeks old. The availability of Cq values for the samples (Figure 4A) provided additional granularity. Thus, samples with N2 Cq <35 performed quite adequately when pooled and the pools tested positive with the Aptima assay. With sample N2 Cq >35, the performance of the Aptima assay including the variability of results progressively deteriorated as the number of viral copies in the pool decreased. In the samples with Cq interval 35–37, the majority of the pools were still

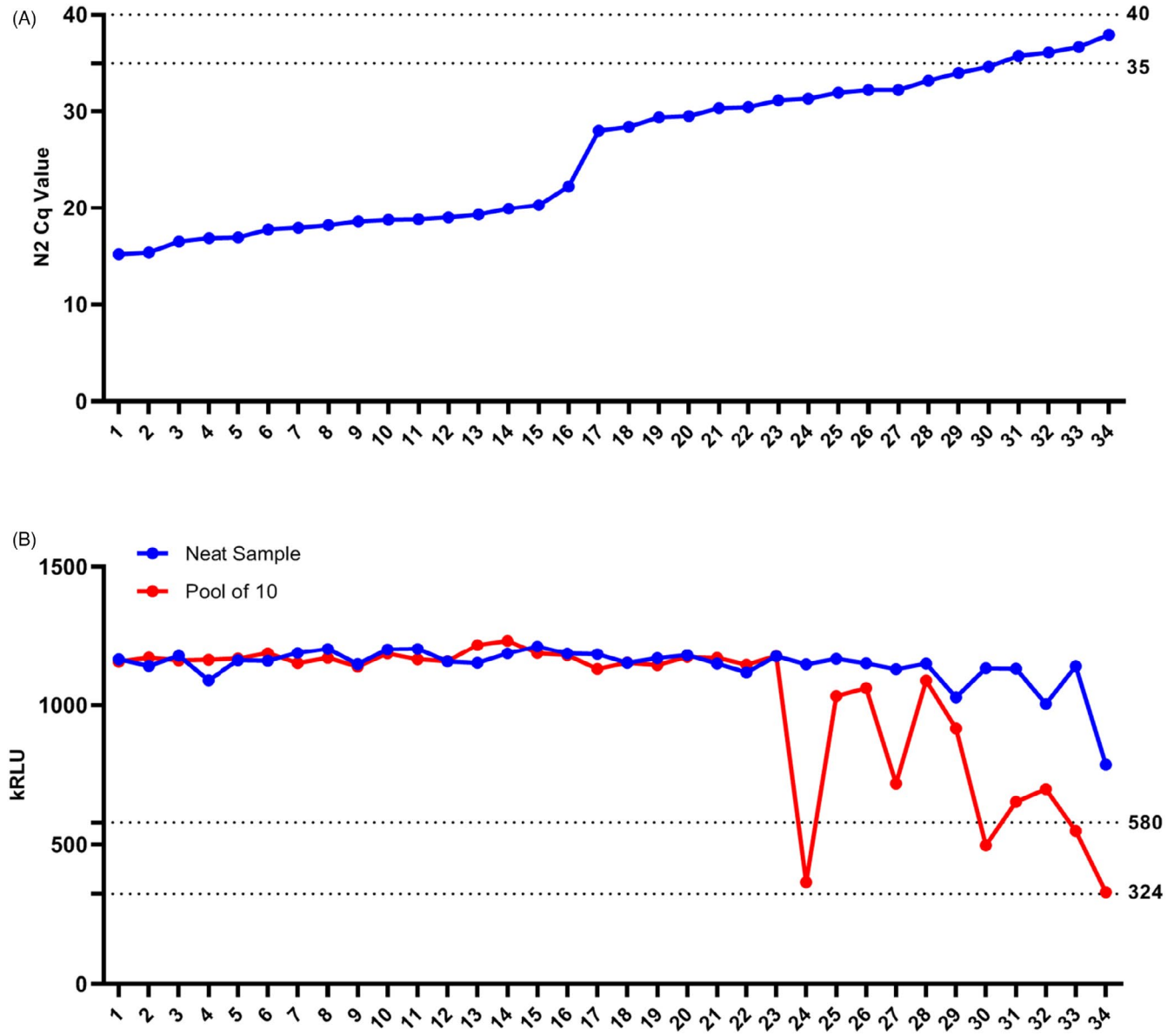


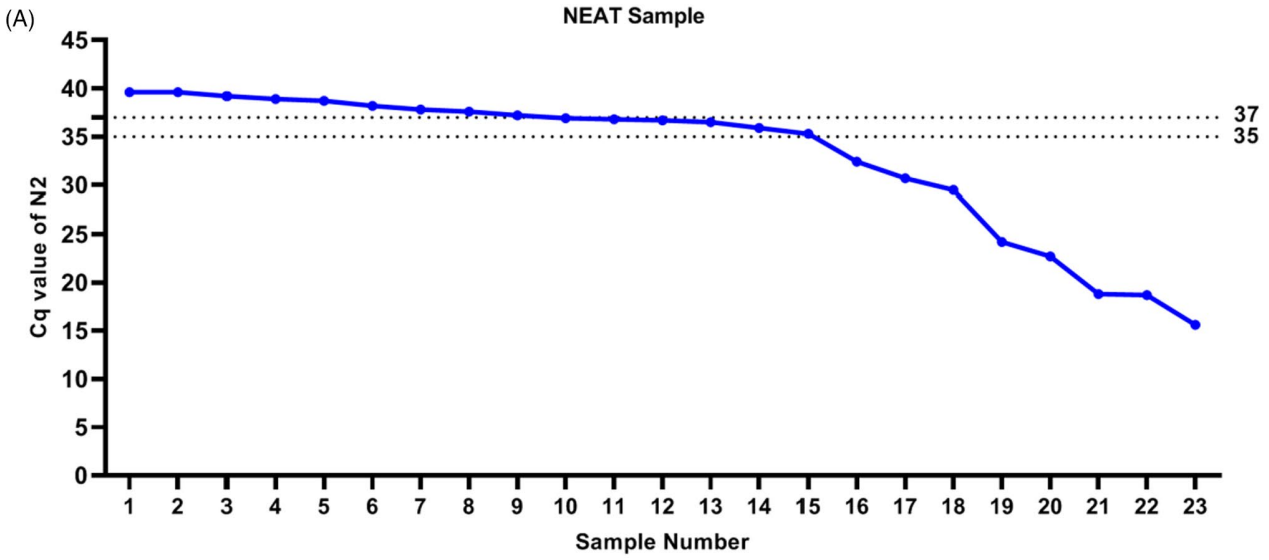
FIGURE 3 An adjusted positivity cutoff also captured weak-positive SARS-CoV-2 samples with high CDC RT-PCR N2 Cq values. 34 SARS-CoV-2-positive neat samples analyzed with the CDC RT-PCR SARS-CoV-2 assay were tested individually (neat) or pooled 10/1 with the Aptima SARS-CoV-2 assay. CDC RT-PCR N2 Cq values of the neat samples are shown in pane (A). Pane (B) shows neat and corresponding pooled results from the Aptima assay. An adjusted positivity cutoff (from 580 for neat samples to 324 for pooled samples) identified pools containing positive samples

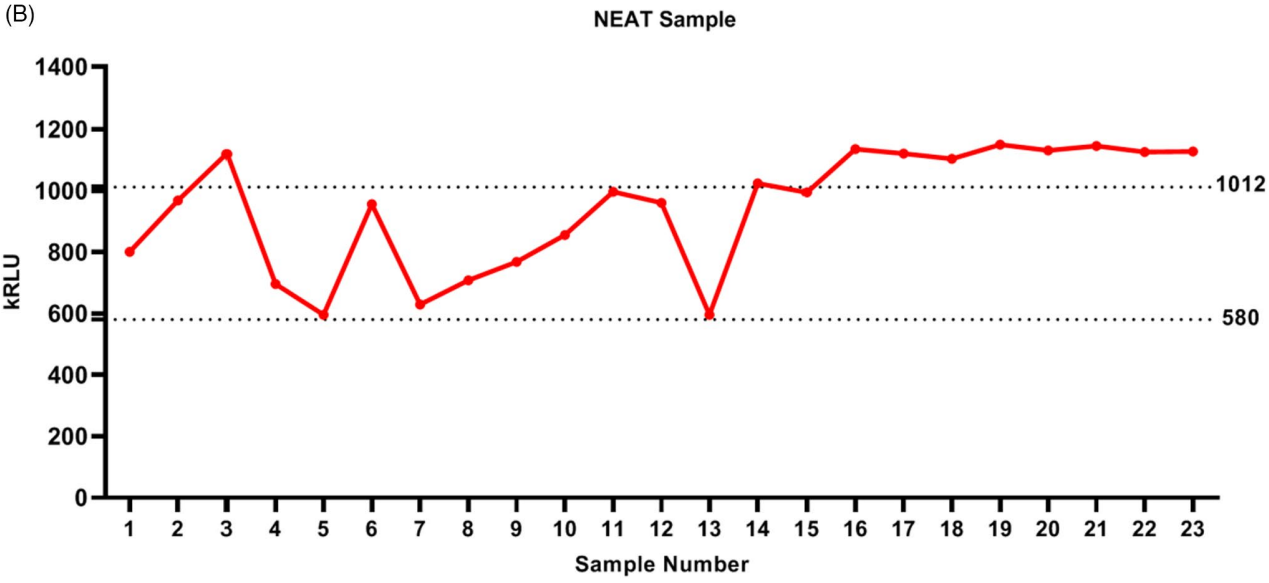
tested positive in all triplicate analyses, while for those samples with Cq >37, the results were erratic. If all 15 samples with N2 Cq >35 are lumped together, then their corresponding pools would test positive with the Aptima assay only in 17/45 = 37.8% analyses.

3.4 | Percentage of false-negative pools

To calculate the PPA of the pooled samples, we combined data on pools tested, shown in Figures 3 and 4. Altogether, we had 57 pools known to contain a positive neat sample (Table 2). We used the pool cutoff for positives at 324 kRLU. Out of the 57 neat positive samples, 42 had kRLU values higher than 1012. Among these 42 samples, 40 corresponding pools tested positive, with RLU >324. The pooled and neat samples tested by the Aptima TMA assay exhibited PPA of 95.2%, with two false-negative results (FNR = 4.76%). For the rest, 15 neat samples with kRLU values lower than 1012, only 6 samples tested positive with PPA of 40%. For all 57 samples combined, the pooled and neat samples exhibited PPA value of 93.4%, higher than the FDA suggested PPA of 85% between pooled samples and individual samples, indicating that the 10/1 pooled sample testing by Aptima TMA assay was an effective screening tool for SARS-CoV-2.

Another way to estimate the percentage of false negatives when pooling 10/1 is to use Cq values from real-time RT-PCR assays run


(A)
NEAT Sample
Cq value of N2
Sample Number
37
35


(B)
NEAT Sample
kRLU
Sample Number
1012
580

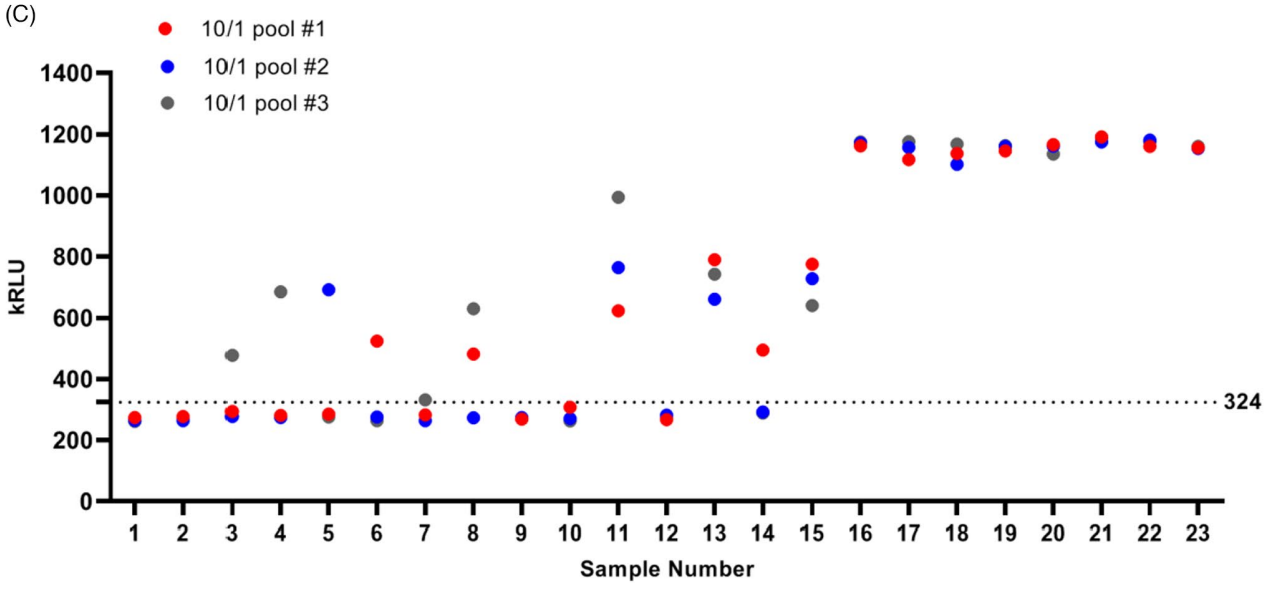

(C)
10/1 pool #1
10/1 pool #2
10/1 pool #3
kRLU
Sample Number
324

FIGURE 4 Aptima SARS-CoV-2 assay triplicates reveal increased variability of results coming from weakly positive samples. An additional 23 SARS-CoV-2-positive samples (neat and pooled) from previous Aptima testing were analyzed in triplicates. (A) The samples were quantitated by the Cepheid SARS-CoV-2 assay immediately before pooling, the graph shows the N2 Cq values for neat samples. (B) Results of the Aptima SARS-CoV-2 assay for the same neat samples tested at the time of presentation. (C) Aptima testing results for 10/1 pools of the samples run in triplicates

on neat samples to see how many of them display weak signal defined as Cq >35. We run routinely high volumes of the CDC real-time RT-PCR SARC-CoV-2 modified assay at UF Path Labs and had data from the assay readily available. Out of 500 positive samples analyzed between July and September 2020, mean sample Cq for the N2 target sequence was 26.817 with SD of 6.794 (Figure 5). Values around Cq of 35 were somewhat overrepresented, as this sample population is fairly mixed and contains results of testing performed also repetitively on already known positive patients with declining viral load numbers resulting in higher Cq. The total percentage of cases exhibiting N2 Cq values over 35 in this sample mix is about 17.8%. The composition of this population is thus somewhat different from when screening symptomatic patients at presentation. Nevertheless, if all these samples were pooled, even with the overrepresentation of weakly positive samples would push the estimated PPA to only 88.9%.

4 | DISCUSSION

With the reopening of schools and university campuses, screening of incoming students and staff for SARS-CoV2 became a critical step to isolate asymptomatic SARS-CoV-2 carriers to reduce the spread of the virus. At a large public university like UF with a student body exceeding 50,000, such screening translates into thousands of samples arriving in the laboratory every day. The sheer volume of samples to be tested requires substantial supply of reagents and consumables that is not always secure. In such circumstances, especially when the prevalence of positives in the tested population is below 1%, pooling of samples to be tested makes sense.

Real-time RT-PCR of a nasopharyngeal swab is the most widely used method for direct SARS-CoV-2 diagnosis.[12,13] However, this method can be extremely labor-intensive depending on the degree of automation available in a particular laboratory. As there is also a shortage of licensed high-complexity clinical lab personnel, an efficient use of existing automated high-throughput instrumentation can ease the demands on manpower.[14] The Hologic Panther instrument is one of such platforms with a theoretical throughput of 1200 samples per day. Their Aptima® SARS-CoV-2 assay was designed for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from NP, nasal, mid-turbinate, and oropharyngeal swab specimens, with a limit of detection (LoD) of 0.01 $TCID_{50}$/ml in the test sample and high clinical agreement (100% PPA, 98.7% NPA) with the validated RT-PCR assay for SARS-CoV-2 RNA.[2] This TMA method is performed in one step at an isothermal condition by targeting the ORF-1ab region of SARS-CoV-2, with a turnaround time (TAT) that is 15–40 min faster than RT-PCR.[15–18] The Aptima assay displays sensitivity comparable with the best RT-PCR methodologies used.[2,3,19]

Testing of samples pooled together at variable ratios has been confirmed as an effective approach to expanding testing capacity, lowering cost per sample, and shortening the TAT[7,10,20,21] However, due to sample dilution after pooling, there is a greater likelihood of false-negative results,[22] especially for positive samples with low SARS-CoV-2 virus copy numbers.[23] Yelin et al. found that one single NP-positive sample with Cq value of 24.5 ± 3.1 could be detected in pools containing up to 32 negative samples, with an estimated false-negative rate of 10%.[6] Farfan et al. set up 5/1 pools using neat positive NP samples showing Cq values from 16 to 36 and concluded that if the Cq value of original neat samples was lower than 35, the pools were still tested positive, with a delta Cq of 2.4–3.4 between the former and the latter. However, if the Cq value of neat positive samples was higher than 35, the pools were more likely to test negative because of the low virus copy numbers.[8] Baccini et al. found that high viral load samples with Cq <30 could be detectable in pools with as many as 29 negative samples, while samples with Cq >37 could be identified only in pools with 4 negative specimens.[24] An important variable to consider when pooling is performed is the pooling ratio, because it impacts the sensitivity of the pooled assay and the maximum ratio depends on the prevalence of positives within the tested population. As the positivity of the tested population varies, the ratio needs to be adjusted accordingly, otherwise pooling would lose its purpose.[25] Abdalhamid et al. found that in a test population with 5% positivity rate, a pool size of 5 samples would provide the largest reduction in the expected number of tests and ratio of 10/1 is optimal for 0.1%–2% positivity rate.[7]

Pooling of samples has been proven to work with RT-PCR,[6–8,26] while there is much less experience about pooling for a TMA assay. It is amenable to pooling exactly like a PCR-based assay with the caveat that the assay results measured in kRLU do not show a strict linear correlation with viral copy numbers when a sample is diluted. We applied pooling to screening a population of asymptomatic UF employees and students returning to campus. With a positivity rate of less than 0.5% we were able to pool 10/1 achieving very considerable savings in reagents, consumables, labor, and shortening the TAT. As an academic lab well-equipped to run NGS and Taqman-based assays, we had already available the instrumentation and human capital needed to accomplish the task. The process of pooling thousands of samples is not trivial and requires both pipetting robot capabilities and informatics support as described elsewhere.[11] The Aptima SARS-CoV-2 assay is highly sensitive, nevertheless, pooling at such high ratio leads inevitably to loss of sensitivity. To improve the sensitivity when pools are tested and to avoid unnecessary false-negative results, we had to set the threshold of 324 kRLU as the positive cutoff threshold for pooled samples when compared with the positive cutoff of 580 kRLU for neat samples. In contrast to RT-PCR, the other aspect of the assay that suffers from low viral copy numbers is the

TABLE 2 57 SARS-CoV-2-positive samples and their 10/1 pools tested with Aptima SARS-CoV-2 Assays

	NEAT	NEA	Pool of 10 #1	Pool of 10 #2	Pool of 10 #3
Sample(#)	(N2 Cq)	(kRLU)	(kRLU)	(kRLU)	(kRLU)
1	20.32	1212	1188	NA	NA
2	18.2	1203	1700	NA	NA
3	18.8	1203	1165	NA	NA
4	18.77	1202	1186	NA	NA
5	17.92	1189	1151	NA	NA
6	19.94	1187	1233	NA	NA
7	22.28	1187	1180	NA	NA
8	28.04	1184	1130	NA	NA
9	29.53	1181	1174	NA	NA
10	16.5	1178	1161	NA	NA
11	31.16	1176	1177	NA	NA
12	29.41	1169	1144	NA	NA
13	31.97	1167	1033	NA	NA
14	15.19	1165	1156	NA	NA
15	16.93	1162	1168	NA	NA
16	17.76	1160	1188	NA	NA
17	19.01	1157	1158	NA	NA
18	19.32	1152	1217	NA	NA
19	28.44	1152	1153	NA	NA
20	32.25	1150	1061	NA	NA
21	30.37	1149	1088	NA	NA
22	33.19	1149	1170	NA	NA
23	18.59	1148	1139	NA	NA
24	31.34	1147	365	NA	NA
25	15.38	1141	1171	NA	NA
26	36.67	1140	549	NA	NA
27	34.66	1133	498	NA	NA
28	35.74	1131	654	NA	NA
29	32.25	1129	719	NA	NA
30	30.46	1117	1145	NA	NA
31	16.85	1089	1163	NA	NA
32	33.99	1028	917	NA	NA
33	36.11	1005	699	NA	NA
34	37.92	787	330	NA	NA
35	24.2	1150	1146	1160	1163
36	18.8	1146	1191	1175	1190
37	32.4	1135	1162	1171	1175
38	22.7	1131	1166	1161	1135
39	15.6	1128	1156	1153	1160
40	18.7	1126	1160	1181	1175
41	30.7	1121	1117	1157	1176
42	39.2	1119	294	278	477
43	29.5	1104	1137	1102	1168
44	35.9	1024	495	292	289

(Continues)

TABLE 2 (Continued)

Sample(#)	NEAT (N2 Cq)	NEA (kRLU)	Pool of 10 #1 (kRLU)	Pool of 10 #2 (kRLU)	Pool of 10 #3 (kRLU)
45	36.8	996	623	764	994
46	35.3	995	775	728	640
47	39.6	968	278	264	272
48	36.7	960	267	282	274
49	38.2	956	524	276	264
50	36.9	856	308	271	263
51	39.6	801	274	265	261
52	37.2	768	269	274	270
53	37.6	708	482	273	630
54	38.9	696	281	274	685
55	37.8	629	283	264	332
56	38.7	596	285	692	276
57	36.5	596	790	661	743

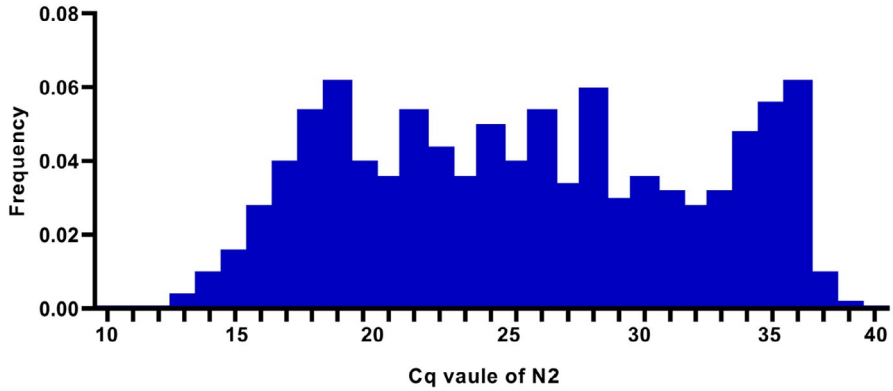


FIGURE 5 Histogram of CDC RT-PCR SARS-CoV-2 N2 Cq value in the tested population. Results for 500 positive samples tested by the CDC RT-PCR SARS-CoV-2-modified assay between July and September 2020 were plotted. The frequency of the corresponding N2 Cq values is shown

precision of measurements resulting in disproportionately increased result variability. The coefficient of variation (CV) of 534 positive neat samples shown in Figure 1 was 6.09%. Once the samples were pooled, the 10/1 pools showed CV of 1.77% for samples with neat N2 Cq <35 and CV of 48.8% for samples with neat N2 Cq >35.

In general, pooling of samples for testing is a trade-off between decreased sensitivity and increased availability of testing. In order to keep the false-negative numbers as low as possible, a sensitive assay like the Aptima SARS-CoV-2 should be used. The composition of the tested sample population is yet another variable that will determine the PPA of the pooled assay. Any samples that are usually associated with low or dwindling viral copy numbers like repetitive testing of known positives in the recovery phase will present a challenge if weakly positive samples are pooled. We estimated the percentage of such problematic samples in two populations tested in our lab. The first one was from our Hologic testing results in May–June 2020 and the second one was from the CDC RT-PCR testing in July–September 2020. Only 3.4% of the Aptima-positives landed in a gray area below mean minus 2 SD corresponding to 1012 kRLU and the positivity cutoff. Pools of exactly those samples performed poorly in the described experiments. From our comparison with the SARS-CoV-2 CDC RT-PCR, 1012 kRLU corresponds to roughly Cq of 35 for N2 target of that RT-PCR assay. However, looking at the RT-PCR-positive samples from July–September 2020, the total percentage of cases exhibiting N2 Cq values over 35 in this sample mix was about 17.8%. In our experiments, about 62.2% of such samples if pooled 10/1, would be resulted as false negative. Therefore, beyond the sensitivity of the assay and pooling ratio, it is also important to know what kind of samples is being pooled, what is the percentage of weakly positives. In the above-described populations, the PPA will be 88.9% with 17.8% weakly positive. If there is only 3.4% weakly positives (like in our neat samples testing on the Hologic platform, Figure 1), the PPA is 93.4%. Studies from the published literature support much lower numbers of weakly positives. Buchan et al. found only 4.29% of samples with Cq >34.5 among 1213 SARS-CoV-2-positive samples from symptomatic patients.[27]

In this study, we show the impact of pooling samples on the performance of the Hologic Aptima SARS-CoV-2 assay. We show that the results of the pooled assays depend on the sensitivity of the assay employed, that 10/1 pooling can be done at low prevalence of positives and the results are negatively impacted by increased numbers of weakly positive samples. All of the results suggest that

10/1 pooled sample testing by the Aptima TMA assay is an effective method for screening saving valuable resources.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All the data generated during and/or analyzed during the current study are available from the corresponding author (PS) upon reasonable request.

ORCID

Petr Starostik https://orcid.org/0000-0002-8992-1230

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