EMERGENCY USE AUTHORIZATION (EUA) SUMMARY OF THE POPLAR SARS-COV-2 TMA POOLING ASSAY
(Poplar Healthcare)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The Poplar SARS-CoV-2 TMA Pooling assay will be performed at Poplar Healthcare, located at 3495 Hack Cross Rd., Memphis, TN 38125, that is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a and meets the requirements to perform high-complexity tests, as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The Poplar SARS-CoV-2 TMA Pooling assay is a TMA assay intended for the qualitative detection of nucleic acids from SARS-CoV-2 in clinician-instructed self-collected nasal swab specimens, and clinician-collected nasal, nasopharyngeal, and oropharyngeal swab specimens from individuals who are suspected of COVID-19 by their healthcare provider.

This test is for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to seven of the individual upper respiratory swab specimens (nasopharyngeal, nasal, or oropharyngeal swabs) that were collected using individual vials containing transport media from individuals suspected of COVID-19 by their healthcare provider. Negative results from pooled testing should be reported as presumptive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive, inconclusive, or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information

Testing is limited to Poplar Healthcare, located at 3495 Hack Cross Rd., Memphis, TN 38125, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet the requirements to perform high complexity tests.

Testing with the Poplar SARS-CoV-2 TMA Pooling assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of TMA assays. The Poplar SARS-CoV-2 TMA Pooling assay is only for use under a Food and Drug Administration's Emergency Use Authorization.

Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

LIMITATIONS

For *in vitro* diagnostic use only.
For prescription use only.
For use under Emergency Use Authorization (EUA) only.
Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Poplar SARS-CoV-2 TMA Pooling assay is in vitro diagnostic test intended for qualitative detection of RNA from the SARS-CoV-2 virus isolated and purified from pools of seven or less of equal volumes of upper respiratory tract swab specimens obtained from individuals who meet COVID-19 clinical and/or epidemiological criteria. Pooled samples are tested on the Poplar SARS-CoV-2 TMA Pooling assay which uses the FDA authorized Aptima SARS-CoV-2 assay from Hologic.

Negative Pools are reported as negative. Positive pools are re-run as individual samples to determine the final results.

INSTRUMENTS USED WITH TEST

The Poplar SARS-CoV-2 TMA Pooling assay is run on the Panther System. Specimens may be pooled via manual pipetting or using a Tecan Evo liquid handling system.

CONTROLS TO BE USED WITH THE POPLAR SARS-COV-2 TMA POOLING ASSAY

The controls used in the Poplar SARS-CoV-2 TMA Pooling assay are described below.

a) A "no template" (negative) control (NTC) is needed to control for run validity and monitors for contamination, assay processing, amplification, and detection. The negative control consists of Specimen Transport Media (STM).

b) A positive template control (PC) is needed to control for run validity and monitors for assay processing, sample extraction, amplification, and detection. The positive control is comprised of two independent in vitro transcripts (IVT) encoding Amplification Region 1 and Amplification Region 2.

c) An internal control (IC) is a non-target nucleic acid added to each reaction and is co-extracted and co-amplified with the target nucleic acid. The IC controls for extraction, amplification and detection in every reaction, reagent integrity, equipment function, and monitors for the presence of inhibitors.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Poplar SARS-CoV-2 TMA Pooling assay Controls – Positive, Negative, and Internal:

NTC – negative for all targets detected
PC – positive for SARS-CoV-2 targets
IC – positive for the internal control in each sample tested

If any control does not perform as described above, the run is considered inconclusive and all specimens are repeated from extraction step.

Examination and Interpretation of Patient Specimen Results:

The interpretation of results for the Poplar SARS-CoV-2 TMA Pooling assay are described in Table 1.

Note on IC: all clinical samples should yield positive results for IC. Samples that fail to show detection of RP within this range and SARS-CoV-2 targets should be repeated from extraction step. If sample detects the SARS-CoV-2 targets, the lack of amplification of IC target can still be valid.

Table 1. Aptima SARS-CoV-2 Results Interpretation

Validity	Positivity	Results Call
Valid	Positive	SARS-CoV-2 Positive
Valid	Negative	SARS-CoV-2 Negative
Invalid	Invalid	Invalid

POSITIVITY RATE

An analysis of all 2,808 specimens collected between July 1 and July 11, 2020 indicated a positive rate of 1.28% (36 positive samples). This supports a 7-sample pooling strategy

with an efficiency of 4.37 at a positivity rate of 1.28%, which is greater than the efficiency for all smaller pool sizes at the same positivity rate.

PERFORMANCE EVALUATION

Poplar SARS-CoV-2 TMA Pooling Assay Analytical and Clinical Performance:

The Poplar SARS-CoV-2 TMA Pooling assay is performed on the Hologic Aptima SARS-CoV-2 assay using pooled individual upper respiratory swab specimens (nasopharyngeal, nasal, or oropharyngeal swabs) that are collected using individual vials containing transport media from individuals suspected of COVID-19 by their healthcare provider.

The analytical and clinical performance of the Hologic Aptima SARS-CoV-2 assay has been demonstrated by Hologic in the Emergency Use Authorization submission authorized on 05/14/2020. The details of the performance of the authorized Hologic Aptima SARS-CoV-2 assay can be found here: https://www.fda.gov/media/138096/download.

Hologic granted Right of Reference to Poplar Healthcare for Hologic's authorized Hologic Aptima SARS-CoV-2 assay.

Clinical Evaluation - Pooling:

Clinical Performance of the Poplar SARS-CoV-2 TMA Pooling assay Using Samples Collected from the Intended Use Population:

To support 7-sample pooling using the Poplar SARS-CoV-2 TMA Pooling assay, 385 nasal swab specimens (362 negative specimens and 23 positive specimens), were combined into pools of 7. Twenty-three positive pools were prepared by combining equal volumes of one positive sample and six randomly-selected negative samples. Thirty-two negative pools were prepared by combining equal volumes of the remaining 224 negative samples into pools of seven. The 55 sample pools were tested using the Poplar SARS-CoV-2 TMA Pooling assay. All 23 positive samples, tested individually, remained positive when tested in 7-sample pools. All 32 pools, expected to be negative, remained negative. The results are summarized in Tables 2 and 3.

Table 2. RLU Values from Positive Samples Tested Individually and in 7-sample Pools

	RLU x 1000	
Sample number	Individually-tested	tested in 7-sample pools
1	1146	1183
2	1105	1181
3	1133	1167

4	1165	1155
5	1095	1216
6	1135	1216
7	1137	1191
8	1167	1189
9	1154	1160
10	1157	1185
11	1140	1201
12	1171	1181
13	1109	1164
14	1196	1163
15	1132	746
16	1148	1182
17	1142	1196
18	1157	1208
19	1126	1188
20	1153	1224
21	1132	1207
22	1156	1168
23	1138	1169

Table 3. Clinical Performance of 7-sample Pooling on the Poplar SARS-CoV-2 TMA Pooling assay		**Results, Individually-Tested**	
		Positive	**Negative**
Results, 7-Sample Pooling	**Positive**	23	0
	Negative	0	25

Performance of 7-sample pooling on the Poplar SARS-CoV-2 TMA Pooling assay against the results from individually-tested samples, consecutively-selected on the Poplar SARS-CoV-2 TMA Pooling assay:

Positive Percent Agreement 23/23 = 100% (95% CI: 85.7% - 100%)
Negative Percent Agreement 25/25 = 100% (95% CI: 86.7% - 100%)

Clinical Performance of the Poplar SARS-CoV-2 TMA Pooling assay Using Low Positive Samples:

In order to demonstrate that the Poplar SARS-CoV-2 TMA Pooling assay retains the ability to detect low positive samples in 7-sample pools, 14 nasal specimens were tested individually and in pools of 7 (consisting of one positive and six negative samples) on the Poplar SARS-CoV-2 TMA Pooling assay.

In order to verify that these fourteen samples were low positive for the Poplar SARS-CoV-2 TMA Pooling assay, these samples were also tested by the cobas SARS-CoV-2 Assay, and Ct values from this assay were analyzed to assess whether these samples can be considered low positive samples. The results of this analysis showed the mean and standard deviation of Ct values for these specimens were mean Ct = 33.0; SD = 1.2 and mean Ct = 34.6; SD = 1.4 for target 1 and target 2, respectively. These mean Ct values are 0.80 greater and 1.81 less than the mean Ct values reported for the cobas SARS-CoV-2 assay LoD (lowest concentration at ≥ 95% hit rate) for target 1 and target 2, respectively.

All 14 positive samples, tested individually by the Poplar SARS-CoV-2 TMA Pooling assay, remained positive when tested in 7-sample pools. The results are summarized in Table 4.

Table 4. Results of Low Positive Samples, as Characterized by the Cobas SARS-CoV-2 Assay, Tested Individually and in 7-Sample Pools on the Poplar SARS-CoV-2 TMA Pooling Assay

	cobas SARS-CoV-2 Assay, Ct		Poplar SARS-CoV-2 TMA Pooling assay, RLU x100	
Sample number	Target 1	Target 2	Individually-tested	Pooled
1	34.29	36.63	1148	746
2	32.48	34.24	1158	1061
3	33.66	36.43	1160	1154
4	31.78	33.43	1177	1185
5	33.62	35.47	721	656
6	33.81	35.7	777	729
7	33.1	32.2	1160	1168
8	32.14	33.74	1172	733
9	32.73	35.61	1179	727
10	31.01	32.61	1212	1170
11	32.77	34.2	1164	1127
12	32.09	33.67	1182	1152
13	35.99	ND	753	722
14	32.24	35.44	1150	1183

ND = Not Detected

The PPA calculated for this study is 100% (14/14) (95% CI: 78.5% - 100%).

Summary of Clinical Evaluation

For the assessment of pooling, 37 positive samples were tested individually and in pools, and among them, 37% were low positive samples. The overall PPA, calculated for both data sets, is 100% (37/37) (95% CI: 90.6% - 100%). The NPA calculated is 100% (32/32) (95% CI: 89.3% - 100%).

PROTOCOL FOR MONITORING OF SAMPLE POOLING TESING STRATEGIES

A protocol for monitoring of sample pooling is used to ensure that there is not an unacceptable drop-off in sensitivity when testing pooled samples and to ensure that the pooling strategy remains efficient over time as the positivity rate fluctuates.

PART 1

The percent positivity rate from individual samples, $P_{individual}$, was calculated from 2,808 specimens to be 1.28% (36 positive samples). The percent positivity rate from pooled samples, P_{pools}, will be updated daily using a moving average of the data from pooled samples from the previous 7-10 days. If P_{pools} is less than 80% of $P_{individual}$ ($P_{pools} \leq 0.8 \cdot P_{individual}$), then a study to re-assess pooling should be conducted (see Part 2 below).

PART 2

Study for Re-Assessment of Pooling

A study for the re-assessment of pooling, as described below, should be conducted monthly or as determined by the criteria in Part 1, whichever is more frequent. To perform a re-assessment study:

1) Samples should be tested individually until 20 consecutive positive samples have been collected. The total number of samples, tested individually, will depend on the positivity rate.
2) Those samples with positive results, when tested individually, will each be pooled with 6 randomly selected negative samples. The resulting 20 pools, each consisting of 1 positive sample and 6 negative samples, should be tested.
3) Calculate Positive Percent Agreement (PPA) as the percent of the positive pools among 20 pools containing a positive sample. If the PPA is less than 85%, reduce the pool size to compensate for lost sensitivity or cease pooling patient specimens.
4) If the pool size is reduced, the 20 positive samples will be tested again (if sufficient volume remains), as 1 positive sample and n-1 negative samples (where n is the reduced pool size). Return to step (3) to calculate the PPA.

PART 3

After the first 5 rounds of the study for re-assessing of pooling has been completed, calculate Positive Percent Agreement (PPA) as the percent of the positive pools among the 100 pools. If the calculated PPA is ≥ 95%, report negative results as “negative”.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Poplar Healthcare, located at 3495 Hack Cross Rd., Memphis, TN 38125, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet the requirements to perform high complexity tests;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and

- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.