Pooled Sample Workflow for the Aptima® SARS-CoV-2 Assay on the Panther® System and Panther Fusion® System

For US Distribution

US only: This workflow for the test has not been reviewed by the FDA. This workflow is being distributed in accordance with Section IV.C. of the FDA's policy for diagnostic tests for Coronavirus disease – 2019 during the public health emergency at *https://www.fda.gov/media/135659/download [fda.gov]*.

Purpose

The purpose of this Customer Technical Bulletin (CTB) is to introduce additional workflow options which allow the testing of pooled samples with the Aptima SARS-CoV-2 assay on the Panther system and Panther Fusion system.

The following information is included in this CTB:

A. Implementation Information
B. Intended Use
C. Warnings and Precautions
D. Implementation, Preparation and Processing of Sample Pools
E. Panther System Test Procedure
F. Interpretation of Results
G. Assay Performance
H. Monitoring

Scope

To accommodate the high demand for COVID-19 testing and the significant need to conserve resources, Hologic is providing our customers with workflow options that allow for the use of pooled samples for testing with the Aptima SARS-CoV-2 assay on the Panther system and Panther Fusion system.

Sample pools may contain up to five individual upper respiratory specimens collected under observation using individual vials containing transport media from individuals suspected of COVID-19 by their health care provider.

This CTB will be distributed upon request and is intended for site administrators, laboratory supervisors, and users of the Aptima SARS-CoV-2 assay on the Panther system and Panther Fusion system.

This CTB will serve to provide all information and instructions associated with implementation, performance, and testing of pooled samples with the Aptima SARS-CoV-2 assay.

Information contained in this CTB will be incorporated into a future revision of the Aptima SARS-CoV-2 assay package insert.

What is Affected

In response to limited customer resources, Hologic has provided alternative workflow options that allow for pooling of samples as a solution to meet the continuous high demand for COVID-19 testing with the Aptima SARS-CoV-2 assay.

A. Implementation Information

A Hologic representative will be in contact to discuss and assist with customer adoption of preparing and testing with pooled samples for the Aptima SARS-CoV-2 assay.

- Pooling of specimens may be performed using the capped workflow or the uncapped workflow for testing with the Aptima SARS-CoV-2 assay.
- Customers will be responsible for tracking, deconvolution, and reporting pooled sample test results.
- No software upgrades are required to begin testing with pooled samples for the Aptima SARS-CoV-2 assay.
- No additional verification testing activities are required by Hologic to begin testing with pooled samples for the Aptima SARS-CoV-2 assay.
- No additional operator proficiencies are required by Hologic, for operators who have previously been deemed proficient to test with the Aptima SARS-CoV-2 assay.

Refer to the package insert for detailed information associated with the Aptima SARS-CoV-2 assay.

B. Intended Use

The Aptima SARS-CoV-2 Assay is a target amplification nucleic acid probe *in vitro* diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/aspirate or nasal aspirates obtained from individuals who meet COVID-19 clinical and/or epidemiological criteria. The Aptima SARS-CoV-2 Assay is for use only under Emergency Use Authorization (EUA) in the US laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to five individual upper respiratory swab (nasopharyngeal, nasal, mid-turbinate, or oropharyngeal swabs), nasopharyngeal wash/aspirate or nasal aspirates specimens that are collected under observation using individual vials containing transport media from individuals suspected of COVID-19 by their healthcare provider. Negative results from pooled testing should not be treated as definitive. If patient's clinical signs and symptoms are inconsistent with a negative result or if results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive 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. For specific patients, whose specimen(s) were the subject of pooling, a notice that pooling was used during testing must be included when reporting the result to the clinician or healthcare provider.

C. Warnings and Precautions

- Contamination may occur if carryover of samples is not adequately controlled during sample pool preparation, handling, and processing.

- Testing of pooled specimens may impact the detection capability of the Aptima SARS-CoV-2 assay and impact sensitivity.

D. Implementation, Preparation and Processing of Sample Pools

Pools of up to 5 specimens may be tested with the Aptima SARS-CoV-2 assay. The pooling size implemented by a laboratory needs to be based upon the desired efficiency of the pooling workflow, the prevalence of infection in the testing population, and the prevalence of low positive specimens near the Limit of Detection of the assay.

Table 1 below provides the maximal efficiency of pooling in relation to positivity, assuming optimal accuracy of the test. For example, a population with a positivity rate of 5% will gain a maximum efficiency of 2.35 if a pool size of 5 samples is implemented, meaning 2.35 more patients can be tested with pooling in comparison to individual testing.

Table 1: Maximal efficiency of pooling in relation to positivity

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ ***(n corresponding to the maximal efficiency)***	***Efficiency of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)***
1%	11	5.11
2%	8	3.65
3%	6	3.00
4%	6	2.60
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

Reference:

EUA Molecular Diagnostic Template for Commercial Manufacturers (updated July 28, 2020)

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

All respiratory specimens currently approved for use under the Emergency Use Authorization of the Aptima SARS-CoV-2 assay may be tested with sample pooling. This includes nasopharyngeal, oropharyngeal, mid-turbinate and nasal swab specimens collected into VTM/UTM, saline, Liquid Amies and Specimen Transport Media (STM) and nasopharyngeal wash/aspirate or nasal aspirate specimens. Only specimens collected into a single type of media may be combined for each sample pool. For example, specimens collected in VTM/UTM should not be combined into a pool with specimens collected in Liquid Amies. Additionally, both neat specimens (those not prepared with STM for testing) and specimens prepared with STM for testing may be included in sample pooling. Each sample pool must be comprised of only neat or only STM prepared specimens. Recommended sample pooling workflow options for different specimen types are provided below.

For Specimens Collected in VTM/UTM, Saline or Liquid Amies

Customers may choose from one of the following two options to perform specimen processing for pooled samples using Specimen Lysis Tubes with the Aptima SARS-CoV-2 assay.

Note: *Hologic testing was performed using pooled samples generated from samples collected in a single collection medium type (e.g., only VTM/UTM or only saline or only Liquid Amies). Combination of multiple transport media types in a single pool has not been evaluated.*

Option 1:
Specimen Preparation Instructions for Neat Samples Pooled Directly into a Specimen Lysis Tube (Hologic SLT, Custom SLT, and Panther Fusion SLT)

Perform the following procedure when pooling specimens collected in VTM/UTM, saline, or Liquid Amies by transferring samples directly into a Specimen Lysis Tube (Hologic SLT, Custom SLT, or Panther Fusion SLT).

A. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. The required specimen to STM ratio in the assay test sample must be maintained for sample pooling. For example, if a pool size of 5 specimens is being utilized, 100 µL of each individual specimen (500 µL total) is required.

B. Uncap the Specimen Lysis Tube and retain the cap.

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the specimen collection container to the Specimen Lysis Tube.

D. Ensure homogenous mixing of each prepared sample pool.

E. Refer to the Aptima SARS-CoV-2 assay package insert for instructions on processing the prepared specimens with the assay.

F. Retain the individual specimens for additional testing, if required.

Option 2:
Specimen Preparation Instructions for Samples Pooled Prior to Transferring to a Specimen Lysis Tube (Hologic SLT, Custom SLT, and Panther Fusion SLT)

Perform the following procedure when pooling specimens collected in VTM/UTM, saline, or Liquid Amies, by pooling the samples prior to transferring into a Specimen Lysis Tube (Hologic SLT, Custom SLT, or Panther Fusion SLT).

A. Obtain a generic empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. The required specimen to STM ratio in the assay test sample must be maintained for sample pooling. The minimum combined volume of individual specimens pooled prior to transferring to an SLT is 500 µL. The same volume of each specimen included in the pool needs to be used.

   Note: *it is recommended to prepare > 500 µL of the specimen pool to account for the potential volume loss associated during* transfer *to the SLT.*

C. Carefully transfer the determined volume of each individual specimen from the specimen collection container to the generic empty tube.

D. Uncap the Specimen Lysis Tube and retain the cap.

E. Prior to testing on the Panther system, carefully transfer 500 µL of the combined specimen mixture from the generic tube to the Specimen Lysis Tube.

F. Ensure homogenous mixing of each prepared sample pool.

G. Refer to the Aptima SARS-CoV-2 assay package insert for instructions on processing the prepared specimens with the assay.

H. Retain the individual specimens for additional testing if required.

For Specimens Collected in Aptima Multitest Transport Tubes

Specimen Preparation Instructions for Samples Pooled Directly into a Generic Tube

Perform the following procedure when pooling specimens collected in Aptima Multitest Transport Tubes by transferring individual specimens directly into an empty tube per specifications in the Panther or Panther Fusion System Operators Manual.

A. Obtain an empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens collected in an Aptima Multitest Transport Tube do not require additional dilution with STM prior to testing.

   Note: *The recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the Aptima Multitest Transport Tubes to the empty tube.

D. Ensure homogenous mixing of each prepared sample pool.

E. Refer to the Aptima SARS-CoV-2 assay package insert for instructions on processing the prepared specimens with the assay.

F. Retain the individual specimens for additional testing if required.

For Specimens Previously Transferred into Specimen Lysis Tubes

Specimen Preparation Instructions for Samples Pooled Directly into a Generic Tube

Perform the following procedure when pooling specimens from Specimen Lysis Tubes (Hologic SLT, Custom SLT, or Panther Fusion SLT) by transferring directly into an empty tube per specifications in the Panther or Panther Fusion System Operators Manual.

A. Obtain an empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens previously transferred into a Specimen Lysis Tube do not require additional dilution with STM prior to testing.

   Note: *the recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the Specimen Lysis Tubes (Hologic SLT, Custom SLT, or Panther Fusion SLT) to the Panther system compatible empty tube.

D. Ensure homogenous mixing of each prepared sample pool.

E. Refer to the Aptima SARS-CoV-2 assay package insert for instructions on processing the prepared specimens with the assay.

F. Retain the individual specimens for additional testing if required.

E. Panther System and Panther Fusion Test Procedure

1. Prepare specimens per the *Implementation, Preparation and Processing of Sample Pools* section in this CTB.

2. Perform the Aptima-SARS-CoV-2 assay test procedure as specified in the assay package insert. There are no assay test procedure changes associated with sample pooling.

Note: *For samples transferred to the Hologic Specimen Lysis Tube, Custom Specimen Lysis Tube, Panther Fusion Lysis Tube, or generic tube, to avoid a processing error, ensure adequate specimen volume is added to the tube.*

Note: *When using the Aptima SARS-CoV-2 uncapped tube assay software, remove the cap from the Positive and Negative controls before loading onto the Panther system.*

F. Interpretation of Results

Negative- Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result or if results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive- Specimens with a positive sample pool 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.

Invalid- Specimens with a repeat invalid sample pool result may need to be tested individually prior to reporting a result.

G. Assay Performance

Clinical performance of sample pools consisting of up to 5 individually collected respiratory specimens was evaluated with the Aptima SARS-CoV-2 assay. Performance of nasopharyngeal swab specimens collected in VTM/UTM were evaluated as representative of all respiratory specimens authorized for use with the assay. For the clinical performance study, 45 positive pools were prepared using 1 positive and 4 negative specimens. Additionally, 20 negative pools were included in the evaluation. The evaluation included testing each individual specimen and each sample pool with the Aptima SARS-CoV-2 assay. Since the kRLU values generated with the Aptima assay are not consistently indicative of target concentration, testing was also performed with the Panther Fusion SARS-CoV-2 assay to provide a corresponding cycle threshold (Ct) value for each individual specimen and sample pool.

The 45 positive specimens included in the study were selected to target inclusion into 4 Ct categories, as shown in Table 2 below, based on a compiled distribution of customer data. Positive and Negative Percent Agreement was calculated for the pooled specimen results in relation to the expected, individual specimen result. Calculations were performed to simulate both a ~10% and 25% prevalence of Low Positive (1-2x LoD) specimen pools, as shown in Table 3 and Table 4, respectively. For both Low Positive prevalence groups, 100% agreement between the pooled and individual specimen result was observed for the Aptima SARS-CoV-2 assay.

Table 2: Ct Ranges of Specimens Included

	Concentration Range	Reference Cycle Threshold	Total N in Study (% of total specimen)
1	25% Quartile	(19-21)	10 (22%)
2	Median	(24-26)	15 (33%)
3	75% Quartile	(29-31)	15 (33%)
4	Low Positive*	(34-37)	5 (11%)
5	Negative	N/A	20 (N/A)

*The LoD of the Fusion SARS-CoV-2 assay is ~36 Ct. The Low Positive range represents 1-2 Ct around the assay LoD. The LoD of the Aptima and Panther Fusion assays are comparable.

Table 3: Percent Agreement in a Population with 25% Low Positive Specimen Prevalence

		Expected Result		
		Pos	Neg	Total
Aptima Pooled Result	Pos	20	0	20
	Neg	0	20	20
	Total	20	20	40

	Agreement	95% C.I.
Positive Percent Agreement	100.0%	(83.9% - 100%)
Negative Percent Agreement	100.0%	(83.9% - 100%)
Overall Agreement	100.0%	(91.2% - 100%)

Table 4: Percent agreement in a Population with ~10% Low Positive Specimen Prevalence

		Expected Result		
		Pos	Neg	Total
Aptima Pooled Result	Pos	45	0	45
	Neg	0	20	20
	Total	45	20	65

	Agreement	95% C.I.
Positive Percent Agreement	100.0%	(92.1% - 100.0%)
Negative Percent Agreement	100.0%	(83.9% - 100.0%)
Overall Agreement	100.0%	(94.4% - 100.0%)

H. Monitoring

Before implementing pooling strategies for testing patient specimens, laboratories must use the "Protocol for Monitoring of Specimen Pooling Testing Strategies" to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol, and retain the generated test results data for inspection by the FDA upon request. The protocol follows the FDA recommended guidelines, as summarized below.

Before implementation of pooling

1. Laboratories must evaluate existing test data in the testing population from the previous 7-10 days to estimate the initial positivity rate.

2. After a positivity rate is established. Laboratories can follow Section D *Implementation, Preparation and Processing of Sample Pools* of this customer technical bulletin.

After implementation of pooling

1. Laboratories must continue to test random samplings of patient samples without pooling. This is required to:

- Evaluate the positivity rate and percent of weak positive samples in the testing population.
- Identify differences in positivity rate between those tested individually and those tested through pooling.

2. Calculate the percent of positive results after implementation of pooling using a moving average (such as a rolling average updated daily using data from the previous 7-10 days) to determine whether there is a change in the positivity rates between individual testing and pooled testing.

3. Re-evaluate testing strategy if the moving average of the positivity rate for pooled samples starts trending in a positive or negative direction.

4. When resource availability is sufficient to meet testing demand, the FDA recommends considering whether the risks of reduced test sensitivity with pooling continue to outweigh the benefits of resource conservation.

What is Required

Ensure that appropriate personnel (laboratory, clinic, supply chain/inventory, purchasing, and accounting) are notified of the information documented in this CTB.

If there are any questions or concerns regarding this communication, Hologic Technical Support may be reached at +1 888 484 4747 or +1 858 410 8511, or by e-mail at molecularsupport@hologic.com.

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