verily Covid-19 Testing

Developing a more efficient, scalable COVID-19 diagnostic testing approach.

Introduction

The urgent need for large-scale, high quality diagnosis of COVID-19 infections has resulted in rapid growth of a diverse and confusing landscape of testing options. Verily has conducted a detailed assessment of testing types, including molecular, antigen, and antibody tests. Key evaluation criteria include the accessibility of sample collection kits and ease of collection, end-to-end test runtime, performance, scalability, and reagent availability.

Based on this evaluation, Verily has adopted the ThermoFisher TaqPath COVID-19 Combo Kit EUA test as a core workflow. This test was selected based on the high performance of the assay (limit of detection of 250 copies/mL, 100% specificity and 100% sensitivity on a synthetic sample test), the reliability of an established manufacturer to supply reagents at scale, and ThermoFisher's early emergency use authorization (EUA). The Verily CLIA laboratory has established this workflow in full compliance with the FDA EUA for testing unpooled samples, and in accordance with HIPAA regulations. However, as the demand for testing continues to increase, so has the need to identify scalable, high-throughput testing workflows—particularly for populations where the positivity rate is low.

The demand for even more testing has continued to grow, requiring innovation of new approaches based on pooling of samples. Pooling refers to the process of combining multiple samples into a single test, which results in conservation of reagents when the expected rate of positive detection is low (since a single negative result for a set of samples in a pool can confirm that they are all negative, using a single test). Two key innovations have enabled implementation of a new pooling strategy at Verily. First, by implementing minor changes to the authorized TaqPath workflow, Verily developed a more sensitive version of the original assay, while still leveraging the TaqPath equipment and reagents. This improved sensitivity enabled innovation of a pooled, high-throughput assay for efficiently identifying individuals with active SARS-CoV-2 infection by offsetting the reduction in sensitivity inherent in pooling strategies. By combining a high-sensitivity RT-PCR assay with a rational sample pooling strategy and automation, the Verily CLIA laboratory can quickly and efficiently test thousands of samples every day.

Pooling is an efficient approach to testing

Our workflow starts with upper respiratory (nasopharyngeal, oropharyngeal, mid-turbinate, or nasal) swabs collected in individual tubes containing liquid transport media. A portion of each liquid sample is then assigned to two independent sample pools along with other non-overlapping samples, and each pool is analyzed for the presence of three regions of the viral genome. By comparing the samples shared between pools testing positive for presence of the SARS-CoV-2 virus and using a sample deconvolution algorithm, the original positive sample can be identified quickly and efficiently. Using this pooling approach, positive

samples can typically be identified with ¼ to ½ the number of tests compared to a singleplex SARS-CoV-2 RT-PCR test (**Figure 1**). Pools that test positive are used to identify candidate individual positive samples, which are subsequently confirmed using individual tests. Viral status is reported based on the combined results of the pools and any confirmatory tests.

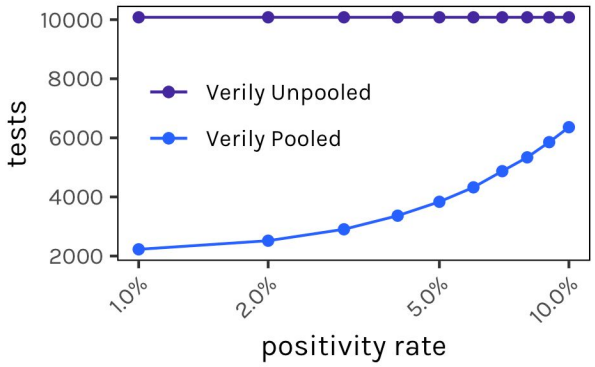

Figure 1. Pooling increases throughput and lowers cost. Simulation of the number of tests required to analyze 10,080 samples through the Verily Pooled and Unpooled methods as a function of population positivity rate. Each point is the mean of 100 simulations.

2D pooling identifies sample status and increases confidence

Verily's 2-dimensional (2D) pooling strategy tests each sample twice to quickly identify potential positive samples and to increase confidence that positive samples are not missed. The pooling strategy begins by arraying samples into a 96 position, 8 x 12 array, followed by construction of 12 pools from the columns and 8 pools from the rows (**Figure 2**). Each sample is a member of two pools and any pair of row and column pools only share one sample. By analyzing the pools, we can determine whether all samples are negative, where a single positive sample is in the array, or, if there are multiple positive samples, identify the subset of samples that are candidate positives, which enables subsequent confirmation by individual re-testing. A conservative algorithm for interpreting the pools defaults to individual re-testing of samples in cases of uncertainty. Notably, all positive samples identified by our pooling strategy are confirmed by individual re-testing to ensure a high level of confidence in the results.

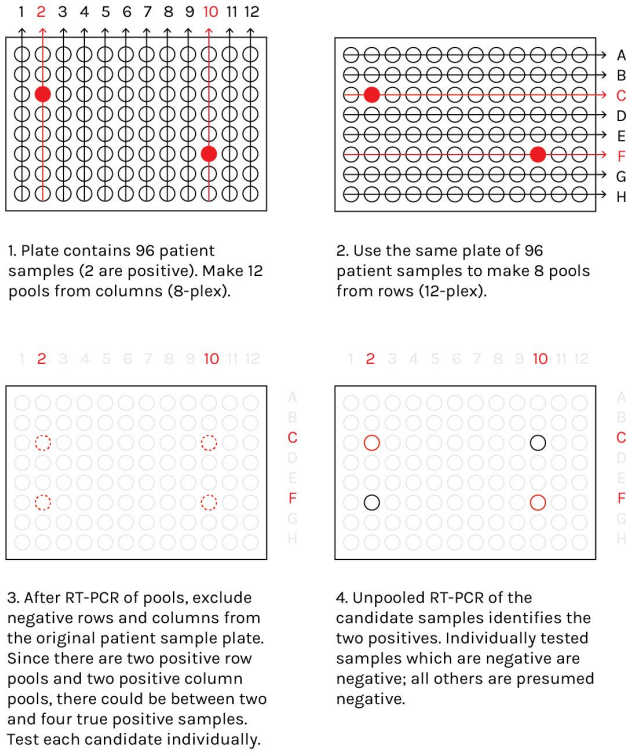

Figure 2. The Verily 2D pooling method.

A pooled test that maintains high sensitivity

A challenge of pooling is that the methods are necessarily less sensitive than their unpooled counterparts, as each pool contains smaller volumes of multiple samples as compared to the entirety of the input consisting of a single sample in the unpooled counterpart test. The larger the number of

samples are pooled, the less sensitive the assay becomes.

The sensitivity improvements implemented in our modified TaqPath workflow, our pooling assay detects samples that many FDA authorized unpooled assays would miss. The limit of detection of Verily's pooled test is 720 genome copy equivalents per milliliter (GCE/mL) of individual sample, a limit of detection more sensitive than the median authorized RT-PCR SARS-CoV-2 test (**Figure 3**). When the Verily-improved sensitivity assay is run in single sample mode, it is among the most sensitive tests, with a limit of detection of 60 GCE/mL. These gains in sensitivity are due to our enhancements to the ThermoFisher TaqPath COVID-19 test.

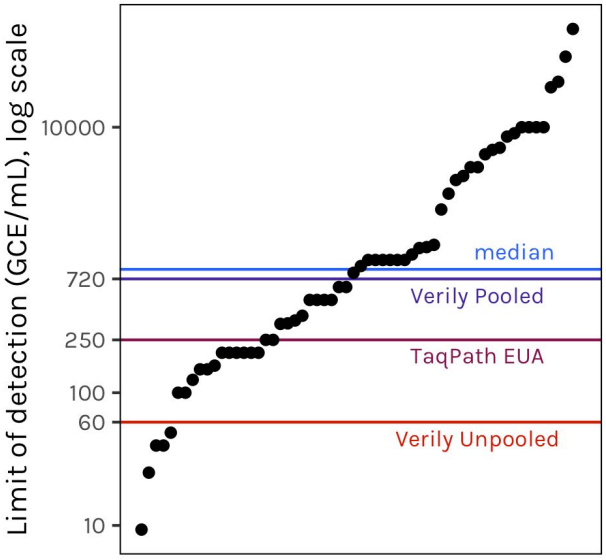

Figure 3. Even when pooled, the Verily COVID-19 RT-PCR Test is more sensitive than the median unpooled test, as of June 30, 2020. Tests shown in plot (63) are those remaining following removal of those that specify LoD in units other than GCE/mL (14) as well as those that did not describe nor utilize a nasopharyngeal or oropharyngeal swab matrix according to the published IFU/EUA summary (10).

A head to head comparison of the Verily high sensitivity unpooled test and the original TaqPath test using 30 unpooled clinical positive and 30 negative upper respiratory swabs shows that our modifications increase the sensitivity of the assay while maintaining diagnostic accuracy (**Figure 4**).

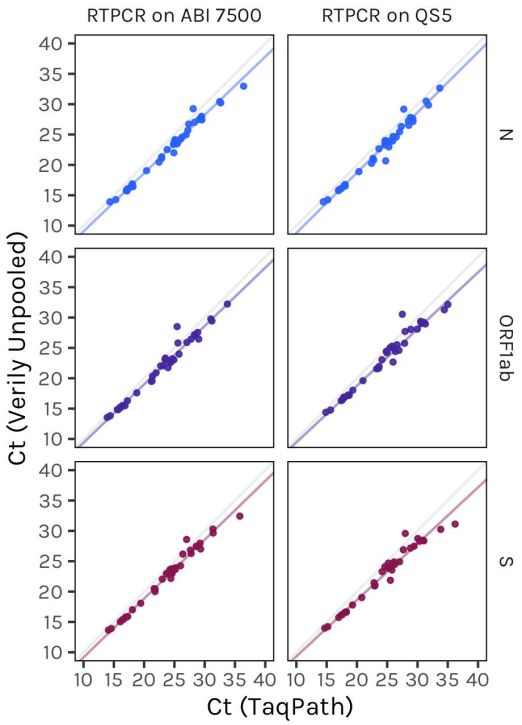

Figure 4. Verily optimizations to TaqPath assay increase sensitivity (colored Passing-Bablok regression line is below the grey y = x line, indicating greater sensitivity) and maintain linearity throughout the range of the assay for all three genomic regions (N, ORF1ab, and S) and on the Applied Biosystems 7500 Fast Dx and QuantStudio 5 RT-PCR instruments.

To evaluate the clinical performance of the Verily high sensitivity test on pooled clinical upper-respiratory samples, a series of clinical sample pools were tested. This included testing

pools consisting of one positive sample with eleven negative samples (a row pool) and one positive with seven negative samples (a column pool). The positive samples were selected to represent a wide range of possible viral loads (**Figure 5**). The increased sensitivity of the Verily test enables detection of samples containing low levels of virus, even when pooled.

The Verily test retains 100% (95% CI: 94.8% to 100%) sensitivity and specificity when run pooled or unpooled and compared to the unmodified TaqPath EUA assay.

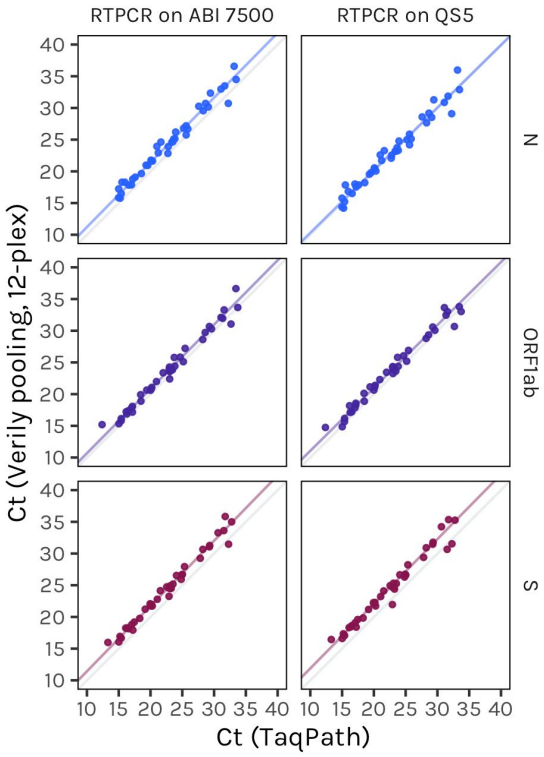

Figure 5. Verily optimizations to TaqPath assay limit the sensitivity loss due to pooling (colored Passing-Bablok regression line is near the grey y = x line, indicating similar sensitivity even when pooling) and maintain linearity throughout the range of the assay for all three genomic regions (N, ORF1ab, and S) and on the Applied Biosystems 7500 Fast Dx and QuantStudio 5 RT-PCR instruments.

Confident identification of positive samples

Through wet lab testing and simulations, we have tested our deconvolution and sample identification strategy and demonstrated its ability to correctly resolve batches of entirely negative samples, batches with just one positive sample, and batches with multiple positive samples. In both our simulations and wet lab testing, all samples were correctly identified, without false positives or negatives.

When a set of 96 samples includes zero or one positive sample, the positive sample can be uniquely located since all samples are in two pools and any pair of row and column pools only share one sample. This sample is re-tested individually, to confirm positivity and proper pooling. Samples that are not positive are presumed to be negative.

When there is more than one positive sample in a set of 96 samples, then the row and column pools reveal a set of possible positive samples. These are re-tested individually to determine which are truly positive.

The Verily pooled test defaults to individual re-testing if row and column pools result in an unexpected pattern (e.g., a positive column but no positive rows).

Summary

The Verily pooled COVID-19 test is a pooled, high-throughput assay for efficient testing of individuals with active SARS-CoV-2 infection by combining a high-sensitivity RT-PCR assay with 2D pooling and automation.