

Validation of pooled nasal swab testing for SARS-CoV2 Surveillance

Background. To support SARS-CoV2 surveillance in defined populations, pooled testing is a cost-effective and resource-conserving approach. Pooled testing combines upfront sample collection of anterior nares (AN) swabs and creation of pools at source (that is, up to 10 swabs are placed into a single tube at the site of sample collection) (Figure 1).

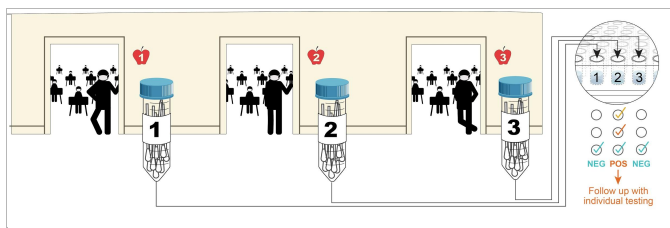


Figure 1. Example of pooled collection in a classroom environment.

The swab tubes are transported to the CRSP laboratory where they are tested using the same highly sensitive PCR test that is being used for individual diagnostic testing. In this version of the test (V3), we look for two targets within the SARS-CoV2 genome (N1 and N2) and one in the human genome (RP) which functions as a test control. These targets are all examined in a single well reaction (Multiplexed RT-PCR) (Figure 2).

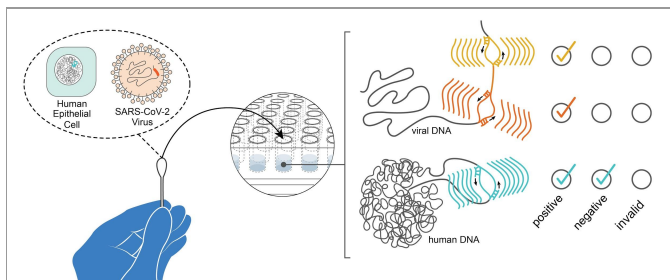


Figure 2. Principle of the assay. The assay tests for two targets within the viral genome and one in the human genome.

In this model of surveillance testing, the intended process is that if a pool test comes back negative then the people whose swabs are in that pool are presumed negative for the purposes of making public health decisions (e.g. remote versus in-person learning). If the pool comes back positive then the people whose swabs are in the pool need to be retested with an individual diagnostic assay.

Design. Surveillance testing for public health purposes is not considered diagnostic testing under FDA and CMS guidelines and is not subject to the same regulatory oversight. However, CDC recommendations are that the testing assay used for surveillance be one that has the quality assurance guarantee that having a clinically validated, EUA assay affords. Although our surveillance test is not an EUA authorized test, it does use the same RT-PCR process as our authorized diagnostic test (V3).

We have performed validation studies in accordance with FDA guidelines for pooled testing. For swab pooling, FDA recommends two main types of validation studies to add pooled testing to an existing validated RT-PCR assay (per the FDA EUA Template from July 28th, 2020). Incorporating these, we present here summaries of four validation analyses we have performed to establish the performance of our multiplexed assay and our pooled test that leverages the assay:

1. Analytical sensitivity and clinical sample concordance for multiplexed version of the RT-PCR assay.
2. Impact of pooling on limit of detection (LOD) of multiplexed assay.
3. Agreement (between pooled and unpooled positive swabs) of the maximum number of swabs with an analyte concentration of 3-5X LoD.
4. Evaluation of the impact of high viral loads on performance of pooled testing.

Results.

Study 1. Analytical sensitivity and clinical sample concordance of CRSP V3 RT-PCR assay

Using a SARS-CoV2 positive specimen spiked into a negative clinical matrix and taken through the entire process, the limit of detection (LOD) (the viral concentration at which 19/20 or 95% of replicates are called) was established at 1600 copies/mL.

Total copies (copies/mL)	# positive / # replicates	Mean N1 Ct (Std Dev)	Mean N2 Ct (Std Dev)	Mean RP Ct (Std Dev)
60 copies (1600 copies/mL)	20/20	32.6 (0.4)	35.8 (0.8)	31.8 (0.3)

Table 1. Limit of detection confirmation of the V3 assay with Sigma rehydration buffer. .

The assay performance was further established through a clinical study of 168 patient specimens. Specimens were run on both the V3 CRSP assay and another EUA authorized RT-PCR assay that has been shown to be high sensitivity through the FDA's Reference Panel program. The acceptable agreement for positive and negative results with a comparator

assay, per FDA, is 95%. Our results demonstrate 98.3% positive agreement and 100% negative agreement with the comparator assay.

		Comparator EUA Assay		
		Pos	Neg	Total
CRSP V3 Assay	Pos	58	0	58
	Neg	1	109	110
	Total	59	109	168
Positive Agreement		98.3% (58/59), 95%CI: (91.0, 99.7%)		
Negative Agreement		100% (109/109), 95%CI: (96.6, 100%)		

Table 2. Concordance of clinical samples with a high sensitivity comparator assay.

Study 2. Impact of pooling on limit of detection (LOD) of multiplexed assay.

In the pooled testing modality, tubes containing up to 10 swabs are received at the laboratory where 5mL of inactivating/rehydrating buffer is added (for comparison, 1mL of the buffer is added to an individual swab in our V2 diagnostic assay). Thus, the dilution factor of the genetic material on each swab is predictable and reproducible, independent of the number of swabs in the tube.

To examine the performance of the RT-PCR assay in pooled samples, a series of 24 spike-in pools were created. In these pools, a confirmed positive clinical specimen of known viral load was spiked on to dry swabs and tested both individually and as part of a maximum capacity pool (that is 10 swabs). The concentration of the spiked virus was chosen to be close to (3-5X) the LOD of the RT-PCR assay.

At these concentrations we observe a reproducible cycle threshold (Ct) shift of 1.6 in the viral (N) targets between a positive called individually and as part of a 10 swab pool (Figure 3). The RP Ct is significantly lower in the pool as expected since human genomic material is present on both positive and negative swabs.

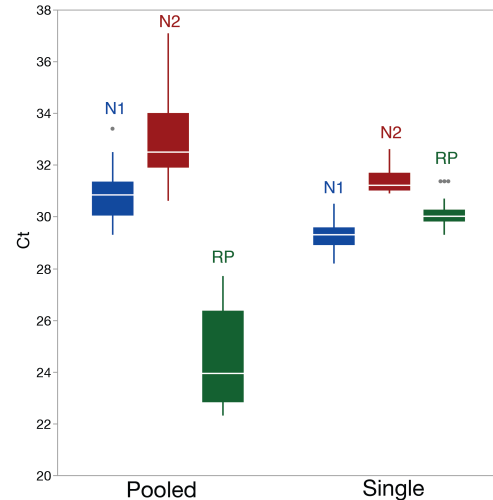


Figure 3. Ct of the N1, N2, and RP targets when assayed individually or as part of a 10 swab pool.

A Ct shift of 1.6 in the viral targets represents a ~5-fold difference in viral copies detected, consistent with the difference in dilution factor between an individual swab and a pool (1mL vs 5mL). Since the individual assay LOD is determined to be 1.6×10^3 copies/mL, we infer that the pooled assay LOD is 8×10^3 copies/mL. For context, the median inferred viral load for a positive specimen tested in the CRSP lab (based on a recent sampling of 10,000 positive specimens) is 6.8×10^5 copies/mL.

Study 3. Agreement between pooled and individual tests close to the LOD of the V3 assay.

The LOD series from Study 2 was assessed for agreement in calls between the pooled and individual testing. The FDA guidelines for pooled testing seek to see at least 95% positive agreement between the testing modalities. We observe 100% positive agreement (Table 3).

		Individual Test		
		Pos	Neg	Total
Pooled Test	Pos	24	0	24
	Neg	0	0	0
	Total	24	0	24
Positive Agreement		100% (24/24), 95%CI: (90.4, 100%)		

Table 3. Concordance of positive samples in pools and individually tested.

Study 4. Impact of high viral loads on performance of pooled testing.

FDA has issued guidance to laboratories to be aware of the potential for assay inhibition by extremely high viral loads. This could occur if multiple highly infected individuals ended up in the same pooled tube.

To study the impact of high viral loads on our V3 assay performance we identified a clinical specimen with a high viral load (N1 and N2 Cts of 10.6 and 11.6 respectively). This individual has an estimated viral load of 1.9×10^9 copies/mL and represents the extreme high end of viral load that we have observed in our testing (less than 0.5% of samples exhibit viral loads in this range) and is >200,000 fold higher than our pooled assay LOD.

To test performance of the assay in the unlikely scenario of three such high viral load individuals in a pool, we spiked this specimen on to individual dry swabs and ran them through the process as part of a 10 swab pool with either one positive (200,000 fold the LOD) or three positive (600,000 fold the LOD) swabs per pool. We repeated this procedure 10 times.

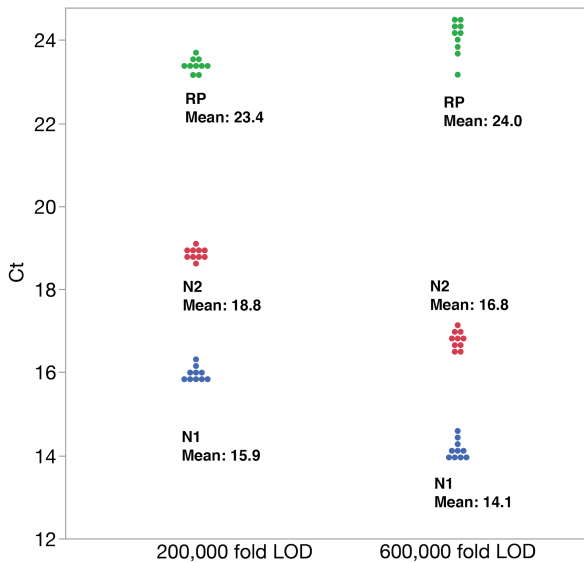


Figure 4. Performance of V3 pooled assay with either one positive or three positive swabs per 10 swab pool.

In 100% of cases the positive pools were called as positive, therefore we saw no evidence of inhibition of our assay based on the extremely high viral loads present in the samples.

Conclusions

1. The CRSP SARS-CoV2 V3 Multiplexed RT-PCR assay is a highly sensitive test with a limit of detection of $\sim 1.6 \times 10^3$ copies/mL of virus.
2. In pooled testing we observe a reproducible dilution of the incoming genomic material that results in a LOD of 8×10^3 copies/mL.
3. Pooled testing of samples close to the LOD of the assay exhibit 100% positive agreement with individual testing of the same samples.
4. High viral loads, up to 600,000 fold the LOD of the assay, do not appear to inhibit the performance of the assay.