**SARS-CoV-2 Pooled Test Performance (Performance of Nucleic Acid Amplification Tests for Detection of SARS-CoV-2 in Prospectively Pooled Specimens)**

**Abbreviations / Important Definitions**

* NAAT = nucleic acid amplification testing
* Ct value = cycle threshold value (indicates how much virus an infected person harbors; CT value is the number of PCR cycles necessary to spot the virus; PCR machines stop running at that point. If a positive signal isn’t seen after 37 to 40 cycles, the test is negative; A test that registers a positive result after 12 rounds, for a CT value of 12, starts out with more than 10 million times as much viral genetic material as a sample with a CT value of 35)[[1]](#footnote-1)
* PPA = Positive percent agreement (calculated using individual testing as the reference method)

**Objective**

* Generally: effect of dilution on analytical sensitivity through sample pooling
* Motivation: potential increase in efficiency gained by pooled testing is offset by a theoretical dilution-related decrease in analytical sensitivity; efficacy varies depending on the performance characteristics of the assay, the prevalence of infection, viral load kinetics, and pooling size and strategy
* Specifically: aimed to evaluate the test performance characteristics of one laboratory-developed and two commercially-available SARS-CoV-2 NAATs on 1648 individual respiratory specimens prospectively grouped in pools of 8 and 4. used these data to validate a stochastic model to estimate optimal pool size, efficiency, and expected positive percent agreement of a two-stage pooled testing algorithm that takes into account prevalence, viral load distribution, and assay analytical sensitivity

**Lab Methods**

* tested 1,648 prospectively-pooled specimens using three different NAATs for SARS-CoV-2: a laboratory-developed real-time reverse transcription PCR (rRT-PCR) assay targeting the *E* gene, and two commercially-available Panther System assays targeting ORF1ab
* Ct result between 40-45 was considered an indeterminate result

**Statistical Methods**

* developed an independent stochastic simulation model to estimate the effects of dilution on PPA and efficiency of a two-stage pooled rRT-PCR testing algorithm, and validated it using these empiric data.
* Assumed independence between samples in same pool
* Input parameters: The modeled PPA estimate is sensitive to the input parameters of 1) proportion of positive tests, 2) assay analytical sensitivity, and 3) viral load distribution
  + proportion of positive tests (*s:* 0.1%, 1.0%, 3.0%, 5.0%, 10.0%, 15.0%)
  + 95% assay limit of detection Ct value (LoD; *l:* Ct corresponding to 95% detection: 32-40)
    - analytical sensitivity of the assay: approximated in this model by the Ct value corresponding to the probability of detecting 95% of true positives, otherwise known as the 95% LoD. Specimens with Ct beyond (???) the LoD are assigned a decreasing probability of detection based on a probit regression curve, the shape of which was determined in the initial validation of the LDT assay
  + percentage of individual amplified Ct values above the LoD (*x:* 5.0%, 10.0%, 15.0%, 20.0%, 25.0%, 30.0%)
    - viral load distribution of the tested population: approximated in this model by the proportion of samples with Ct greater than the LoD. This makes the model output independent of the actual LoD Ct value itself, allowing for the model to be used across different rRT-PCR assays
  + pool size (*p:* 1-20)

**Results**

* Positive percent agreement (PPA) of pooled versus individual testing ranged from 71.7%-82.6% for pools of 8, and from 82.9%-100.0% for pools of 4.
* Our model demonstrated that PPA was dependent on the proportion of positive tests, cycle threshold distribution, and assay limit of detection**.**

Some practical considerations

* pooling in multiples of 4 would be more efficient for the robotic liquid handlers in our laboratory

Very basic understanding question

* Pooling dilutes samples, makes it more difficult to detect positive samples. Larger pools (more dilution) and less prevalent disease (less likelihood of a positive sample) mean you are more likely to have false negatives (lower PPA)
  + Relationship between pool size and PPA:
    - At a given prevalence, increasing pool size decreases PPA
    - When prevalence is high enough, you are more likely to have multiple positive tests per pool. So at high prevalence, increasing pool size increases PPA. But if prevalence is this high then pooling is inefficient (lots of positive pools to deconvolute and retest)
* Ct beyond LoD (i.e. Ct greater than LoD?) means less viral load in a sample, so assay is less sensitive?

1. https://www.sciencemag.org/news/2020/09/one-number-could-help-reveal-how-infectious-covid-19-patient-should-test-results [↑](#footnote-ref-1)