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Implementation of SARS-CoV2 Screening in K–12 Schools Using In-School Pooled Molecular Testing and Deconvolution by Rapid Antigen Test

Nira R. Pollock,[a] David Berlin,[b] Sandra C. Smole,[c] Lawrence C. Madoff,[c] Catherine Brown,[c] Kelsey Henderson,[b] Elizabeth Larsen,[d] Jeremiah Hay,[d] Stacey Gabriel,[e] Atul A. Gawande,[b,f] Niall J. Lennon[e]

[a]Department of Laboratory Medicine, Boston Children's Hospital, Boston, Massachusetts, USA
[b]CIC Health, Cambridge, Massachusetts, USA
[c]Massachusetts Department of Public Health, Jamaica Plain, Massachusetts, USA
[d]Executive Office of Health and Human Services, Massachusetts Department of Health and Human Services, Boston, Massachusetts, USA
[e]Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA
[f]Ariadne Labs, Brigham and Women's Hospital and Harvard TH Chan School of Public Health, Boston, Massachusetts, USA

ABSTRACT Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) testing is one component of a multilayered mitigation strategy to enable safe in-person school attendance for the K–12 school population. However, costs, logistics, and uncertainty about effectiveness are potential barriers to implementation. We assessed early data from the Massachusetts K–12 public school pooled SARS-CoV2 testing program, which incorporates two novel design elements: in-school "pod pooling" for assembling pools of dry anterior nasal swabs from 5 to 10 individuals and positive pool deconvolution using the BinaxNOW antigen rapid diagnostic test (Ag RDT), to assess the operational and analytical feasibility of this approach. Over 3 months, 187,597 individual swabs were tested across 39,297 pools from 738 schools. The pool positivity rate was 0.8%; 98.2% of pools tested negative and 0.2% inconclusive, and 0.8% of pools submitted could not be tested. Of 310 positive pools, 70.6% had an N1 or N2 probe cycle threshold (C_T) value of ≤30. In reflex testing (performed on specimens newly collected from members of the positive pool), 92.5% of fully deconvoluted pools with an N1 or N2 target C_T of ≤30 identified a positive individual using the BinaxNOW test performed 1 to 3 days later. However, of 124 positive pools with full reflex testing data available for analysis, 32 (25.8%) of BinaxNOW pool deconvolution testing attempts did not identify a positive individual, requiring additional reflex testing. With sufficient staffing support and low pool positivity rates, pooled sample collection and reflex testing were feasible for schools. These early program findings confirm that screening for K–12 students and staff is achievable at scale with a scheme that incorporates in-school pooling, primary testing by reverse transcription-PCR (RT-PCR), and Ag RDT reflex/deconvolution testing.

KEYWORDS antigen, pooled, reflex, SARS-CoV2, schools, screening, testing

In March of 2021, the U.S. Department of Health and Human Services announced an investment of $10 billion under the American Rescue Plan to increase screening to help schools reopen (1). Pooled testing, a form of group test whereby individual specimens are combined prior to the laboratory test process, increases testing capacity while potentially reducing test costs. Many scientists and epidemiologists have advocated for pooled testing for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) in large population cohorts (2–5). Several strategies for pooled testing have been proposed. The simplest pooling design dates back to the work of

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Address correspondence to Niall J. Lennon, nlennon@broadinstitute.org.

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Robert Dorfman in 1943 (6). If the pool test result is negative, the members of the pool are presumed to be negative. If the pool test result is positive, the constituent members of the pool must be tested individually (known as "pool deconvolution," or "reflex testing") to determine who is actually positive for the test analyte.

The Broad Institute has established a distributed Dorfman pooled testing process, termed "pod pooling," in which the pooling event happens at the site of specimen collection. In this model, individual anterior nasal (AN) swab samples are placed into a single dry sample tube (no transport medium), with a maximum of 10 swabs per tube. At the laboratory, highly automated processes are employed for accessioning, decapping, swab rehydration, sample transfer, RNA extraction, and target analyte detection. The downstream testing for pooled specimens (from extraction onwards) follows exactly the same process as the laboratory's Emergency Use Authorization (EUA) clinical diagnostic reverse transcription-PCR (RT-PCR) for SARS-CoV2 (https://www.fda.gov/media/146499/download).

In order for pod pooling to be effective, four things must be in place: (i) short turnaround times for pooled test results, (ii) pooled test capacity, (iii) rapid reflex testing, and (iv) a robust result reporting system. The Broad Institute testing laboratory operates 24/7 and has a current capacity for pooled tests of 40,000 per day (equating to up to 400,000 individuals per day). The requirement for collection of a new sample for pool deconvolution in this program design led to the idea of using antigen (Ag) rapid diagnostic tests (RDTs) for reflex testing, leveraging a recent evaluation of the performance of the Abbott BinaxNOW COVID-19 Ag card (7) for adults and children (8) and state procurement of this test at the scale required for this use case. On 8 January 2021, the Massachusetts departments of Early and Secondary Education (DESE) and Public Health (DPH) announced a new asymptomatic pooled testing program utilizing this design for Massachusetts schools (9). The goal of this analysis was to evaluate the early operational and analytical data from this program to assess the feasibility and performance of this approach to pooled testing and reflex testing in schools.

MATERIALS AND METHODS

All public schools in Massachusetts were invited to sign up to participate in the DESE/DPH pooled testing program. In order to facilitate school participation in the program, Massachusetts public health officials distributed sample standing orders for school or other local providers and added participating schools as locations under the State Public Health Laboratory's Clinical Laboratory Improvement Amendments (CLIA) Certificate of Waiver. In collaboration with DESE/DPH-contracted vendors, including CIC Health and Project Beacon, each school assembled its own team to execute the pooled testing program, including setting up school/district-specific processes for obtaining parental consent, implementation of pooled sample collection and submission, data management, result reporting and communication with staff families, and reflex testing. Teams typically included school nurses and district nursing leaders, parent volunteers, and, in some cases, other members of the school district (e.g., superintendent); many districts also requested additional assistance from hired support staff (e.g., individuals from health care staffing agencies, emergency medical technicians, or home health care providers) for sample collection. Teachers were sometimes involved as observers in observed self-swabbing (see below).

Testing of pooled swab samples was performed at the Broad Institute using a series of automated steps. First, conical tubes containing dry anterior nasal swabs (Steripack, Lakeland, FL) were decapped on a TekMill 50-ml centrifuge tube handler robot (TekMill, Champaign, IL) which also dispensed 5 ml of a rehydration and lysis buffer (catalog no. L8285-CONF; Sigma-Aldrich, St. Louis, MO). Filled tubes were transferred to a static rack and the rack was shaken on an orbital shaker for 15 min. Hamilton Starlet automation was used to transfer 150 μl of rehydration buffer from each tube to a well of a 96-well microtiter plate. An aliquot of 37.5 μl of rehydration buffer from each well of four 96-well plates was added to one 384-well plate (using an Agilent Bravo liquid handler; Agilent, Santa Clara, CA) prior to RNA extraction. RNA extraction was performed using MagMAx viral RNA extraction reagents on an Agilent Bravo liquid handler. Master mix plates for quantitative PCR (qPCR; 384 well) were created on Tempest automation (Formulatrix, Bedford, MA). The Clinical Research Sequencing Platform (CRSP) SARS-CoV2 RT-PCR assay utilizes two viral probes (N1 and N2) and a single human probe (RP) which are detected using a QuantStudio 7 instrument (Thermo Fisher, Waltham, MA). Results were determined based on the detection of viral and human probes, reviewed by clinical lab staff, and delivered back to pooled testing vendors (CIC Health and Project Beacon) via software integration. Viral load calculations were based on a standard curve created using a SARS-CoV2 construct (Twist Biosciences, San Francisco, CA).

Pooled testing was performed weekly by participating schools. At the pooling site, asymptomatic students and staff were organized into pools of 2 to 10 individuals. Observed self-collection of AN swabs was employed for adults and older students (grades 2 to 12), and younger students were swabbed by

designated staff (either school nurses or hired support staff [as defined above], wearing personal protective equipment [PPE], including gloves, gown, and surgical mask). Schools kept track of which individuals were in each pool, either on paper or with the help of software provided by their DESE/DPH operations vendor. All the swabs in a pool were tested together and the lab reported a single group result to the registrant. If a pool test returned positive, every individual in the pool had to be followed up with an individual (reflex) test, requiring collection of a second swab; while the program did not require individuals to isolate prior to reflex testing, management in this time window varied by school district. Program instructions guided schools to utilize BinaxNOW (with AN swab collected and test performed by trained staff, i.e., school nurses or hired support staff) for positive pool deconvolution, though a minority of schools chose to use individual PCR for pool deconvolution instead of or in addition to BinaxNOW. BinaxNOW deconvolution testing was performed at sites selected by each school/district (e.g., within each school or at a central site within the district), and results were reported electronically to the Massachusetts DPH using operations vendor software. For BinaxNOW deconvolution testing, if all individuals tested negative with BinaxNOW, schools could choose between performing reflex PCR (swab for PCR was collected either in parallel with swab for BinaxNOW or the following day) or a second round of BinaxNOW testing on the following day. Deidentified pool deconvolution data provided voluntarily by schools working with one DESE/DPH operations vendor (CIC Health) were available for this analysis, allowing assessment of the performance of BinaxNOW in this testing scenario (of note, cycle threshold [C_T] values were not available to school districts at the time of testing). This project was reviewed and approved by the Massachusetts DPH Investigational Review Board and was deemed nonhuman subject research.

RESULTS

Between January 2021 and April 2021, pooled AN swab RT-PCR was implemented at schools in Massachusetts, including 738 public schools (including elementary, middle, and high school student populations) submitting testing to the Broad Institute laboratory (Table 1). During this period, 187,957 individuals were tested across 39,297 pools submitted by schools to the Broad Institute with assistance from operations vendors CIC Health (30,191 pools) and Project Beacon (9,106 pools) (Tables 1 and 2). The average number of swabs per pool was 7 (range, 2 to 10). There were 310 positive pools (255 and 55 pools from CIC Health and Project Beacon, respectively), for a positive pool rate of 0.8% (Table 2). The testing platform routinely delivered results within 12 to 16 h from sample receipt, and the median turnaround time from pooled swab collection to result return was 20.7 h (Table 1).

The mean RT-PCR cycle threshold (C_T) values for positive pools were 26.4 and 27.9 for the N1 and N2 viral targets, respectively (Table 2), equating to a mean viral load of 7.5×10^4 copies/ml based on the assay standard curve (see Materials and Methods). Assay validation data had demonstrated that positive pool C_Ts are ~1.6 C_T greater than the individual swab C_T, a finding expected based on the fixed dilution factor used for pools (5 ml of rehydration buffer added to tube regardless of number of swabs) compared to individual swab testing (1 ml added to one swab) (10). Pollock et al. (8), in a large study comparing BinaxNOW and Broad Institute RT-PCRs performed on paired AN swabs from symptomatic and asymptomatic adults and children, reported 95.8% sensitivity of the BinaxNOW (all subgroups combined) when the RT-PCR C_T was ≤30 and 81.2% sensitivity with a C_T of ≤35. Based on the dilution factor C_T shift, 75.5% (234/310) of the positive pools would have been predicted to have an individual swab with an N1 or N2 target C_T of ≤30 and 98.7% (306/310) of pools a sample with a C_T of ≤35. However, the impact of the interval between pooled sample collection and deconvolution testing (expected to range from 1 to 3 days) on viral load—and thus BinaxNOW sensitivity—was unknown.

Between 4 January 2021 and 9 April 2021, of the 310 positive pools (all followed by individual reflex testing for deconvolution, the majority of which was done with BinaxNOW; see Materials and Methods), 124 positive school pools were followed by BinaxNOW testing for deconvolution and had both pooled PCR C_T data and detailed reflex testing results available for analysis of BinaxNOW operational and analytical performance in the reflex testing scenario. Overall, 92/124 (74.2%) BinaxNOW deconvolution attempts yielded a positive BinaxNOW result. In 10/124 pools, not all individuals in the pool were tested by BinaxNOW (due to factors including electing to do PCR elsewhere, development of symptoms, refusal, or quarantine), though in 3/10, the incomplete deconvolution testing nonetheless yielded a positive BinaxNOW result. Deconvolution results for the 114 pools with full BinaxNOW

TABLE 1 High-level metrics of the school testing program as of 9 April 2021[a]

Metric	Value
No. of schools sending pools	738
No. of pooled tests run	39,297
Median no. of swabs per pool	7
Range of no. of swabs per pool	2–10
No. of individuals tested in pools[b]	187,957
Median TAT (hrs) from collection time to result return	20.7
Interquartile range of TAT (hrs)	14–28.4

[a]Numbers are for schools included in the Massachusetts pilot program that were processed for testing at the Clinical Research Sequencing Platform (CRSP) at the Broad Institute. TAT, turnaround time.
[b]Total number of swabs; note that individuals may be tested in more than one pool over time (i.e., repeat testing).

deconvolution are presented in Table 3; of the 114 pools, 89 (78.1%) yielded a positive BinaxNOW result. For each of the five deconvolution outcomes, the percentage of pools with either an N1 or N2 C_T value of $\leq$30 is shown. Of 80 fully deconvoluted pools with either an N1 or N2 C_T value of $\leq$30, 74 (92.5%) identified an individual with a positive BinaxNOW result; of the 310 positive pools detected in the program over this time window, 219 (70.6%) had an N1 or N2 C_T value of $\leq$30. BinaxNOW was performed 1 to 2 days after PCR in 93.0% of pools and within 1 day in 75.4% (range, 1 to 3 days).

An informal survey of school districts participating in the testing program pilot identified a consistent need for additional staff support in a majority of participating schools (e.g., nurses tasked to work on the testing program instead of their normal duties, hired professionals for assistance with pooled sample collection, or couriers) in order to successfully implement the program, given time and personnel requirements for in-school pool collection/sample submission, communication with individuals in positive pools, and reflex testing.

DISCUSSION

This analysis presents operational and analytical data from the first months of the Massachusetts DESE/DPH K–12 school testing program, one of the largest school-based

TABLE 2 Pooled testing results as of 9 April 2021[a]

Metric	Value	%
No. of pooled tests run	39,297	100
No. of negative pools[b]	38,593	98.2
No. of positive pools[c]	310	0.8
No. of invalid pools[d]	31	0.1
No. of inconclusive pools[e]	67	0.2
Unsatisfactory specimens (test not performed)		
Reason: at least one swab upside down in tube	215	0.6
Reason: lab incident	46	0.1
Reason: sample received >56 h after collection[f]	26	0.1
Reason: sample too viscous after rehydration	3	0.01
Reason: tube label data do not match order	2	0.01
Positive pool viral N1 target C_T, mean (SD)	26.4 (5.6)	
Positive pool viral N1 target C_T, range	15.3–37.8	
Positive pool viral N2 target C_T, mean (SD)	27.9 (6.3)	
Positive pool viral N2 target C_T, range	15.7–39.8	

[a]Results are based on a RT-PCR with multiplexed N1, N2, and RP targets as outlined in the assay EUA (EUA200147). C_T, cycle threshold.
[b]Negative pools have no detected viral targets and a positive human control target (RP gene).
[c]Positive pools have two detected viral targets (N1 and N2).
[d]Invalid pools have no detected viral targets and no detected human control target.
[e]Inconclusive pools have only one viral target detected.
[f]Acceptable specimens for testing must be received at the laboratory within 56 h of collection based on FDA stability studies.

TABLE 3 Reflex testing performance

Metric	No. (%)	Pool N1 or N2 C_T value of <30, no. (%)
Positive pools with available reflex test data	124	86/124 (69.4)
Pools with complete BinaxNOW deconvolution (all individuals tested by BinaxNOW)	114/124 (91.9)	80/114 (70.2)
True-positive BinaxNOW[a]	89/114 (78.1)	74/89 (83.1)
False-negative BinaxNOW[b]	5/114 (4.4)	1/5 (20.0)
Likely false-negative BinaxNOW[c]	4/114 (3.5)	1/4 (25.0)
Pool PCR result unconfirmed[d]	12/114 (10.5)	2/12 (16.7)
Unknown[e]	4/114 (3.5)	2/4 (50.0)

[a]Individual in pool tested positive by BinaxNOW (in 88/89 cases, only one positive BinaxNOW result was observed; in 1/89, two were observed [the two individuals were from the same family]).
[b]All BinaxNOW results were negative, but an individual in the pool tested positive by reflex PCR.
[c]All BinaxNOW results were negative, but an individual in the pool had an inconclusive reflex PCR result (only 1 of 2 PCR targets positive).
[d]All BinaxNOW and additional reflex testing results (repeat BinaxNOW testing ($n = 4$) or PCR testing ($n = 8$) on all individuals in the pool) were negative, making it impossible to confirm the positive pool PCR result and leaving open the possibility that the PCR result was a false positive.
[e]All BinaxNOW results were negative, but additional reflex PCR results were not available.

screening programs in the United States. The 3 months of pod pooling data indicate both that this method was operationally feasible in the school setting and that observed positive pool rates were extremely low (0.8%). The distributed pooling model facilitates scale and rapid turnaround and potentially reduces laboratory costs compared to strategies in which sample pooling and reflex testing are performed by the laboratory. The low rate of unsatisfactory incoming samples indicates that users in the school setting can effectively follow provided testing protocols (any failed testing due to unsatisfactory submissions was addressed by follow-up communication with the submitting school). It should be noted that implementation of this model requires attention to staffing needs for in-school sample collection and reflex testing and a commitment from participants to return for reflex testing, which can be challenging for some families and staff.

This pooled testing model requires a feasible and reliable reflex testing strategy that can be deployed as soon as possible after a positive pool is detected. The use of an Ag RDT significantly shortens the period to generate (and act on) an individual-level result.

BinaxNOW pool deconvolution was successful in 92.5% of pools with C_T values of $\leq$30 for either N1, N2, or both targets, which represented 70.6% of positive pools submitted in the program at the time of this analysis. Pools with lower C_T values (corresponding to higher viral titers) and positive BinaxNOW reflex tests are expected to correspond to individuals with culture-positive samples, suggesting higher infectivity (11) (though the mitigation measures in place in the school setting, including masking, distancing, ventilation, and hygiene, would be expected to reduce risk of in-school transmission). BinaxNOW was performed 1 to 2 days after PCR in 93% of pools. As presented in Table 3, only 9 confirmed false-negative or likely false-negative BinaxNOW results were observed out of 114 fully completed deconvolution attempts, and of those, 7 (77.8%) had pool C_T values of $>$30 for both targets. However, given that 15/89 pools (16.9%) with positive BinaxNOW deconvolution results had C_T values of $>$30 for both N1 and N2, and given the benefit of rapid case identification, it may be worthwhile to continue to attempt BinaxNOW regardless of the C_T value of the positive pool. We note that as presented in Table 3, in 12/114 (10.5%) fully deconvoluted pools, two rounds of reflex testing of all individuals in the pool yielded fully negative results, leaving open the possibility that the PCR result was a false positive. However, we also note that 10/12 of those pools had both N1 and N2 C_T values of $>$30, suggesting that the time between pooled testing and reflex testing may have coincided with a decrease in viral load to a level that was no longer detectable. Of note, interpretation of positive

BinaxNOW reflex results as true positives was felt to be justified by the context (positive pool) and the high observed specificity of BinaxNOW (8).

It should be noted that 25.8% of BinaxNOW pool deconvolution testing attempts (in the 124 pools with complete deconvolution data available) did not identify a positive individual, requiring either reflex PCR done within the school program or, in some cases, outside reflex PCR for individuals who did not return for BinaxNOW testing. These early program data help to highlight the logistic complexity of requiring a return visit for reflex testing after a positive pool and the necessity of having a plan for expedited reflex PCR available for all participants. Thus far, performing BinaxNOW tests on two consecutive days if the first round of BinaxNOW testing is negative has not yielded any positive BinaxNOW results. Analysis of reflex results for pools with inconclusive pool PCR results is under way, with the goal of defining optimal management strategies for this result scenario. Finally, optimization to streamline reflex testing procedures, particularly for large districts with student/staff transportation and communication challenges, is in progress.

While this analysis has demonstrated the operational and analytical feasibility of the in-school pod pooling and BinaxNOW reflex testing strategy, both this study and the overall approach have limitations. This study was not designed to assess the overall efficacy of the program in terms of cases or hospitalizations averted or to assess the negative impacts (such as school/classroom closures), and it was also not designed as a cost-effectiveness analysis to assess program utility. These analyses would be of clear value to those deciding on strategies for school screening, and data for such analyses are currently being gathered but are beyond the scope of this article. As noted above, in-school pooled sample collection and reflex testing require a substantial amount of effort, which must, in turn, be factored into program cost along with actual testing costs. The requirement for a second swab collection for pool deconvolution introduces both the possibility of false-negative BinaxNOW results (requiring additional PCR reflex testing and introducing uncertainty) and the operational complexity of obtaining a second swab from individuals who are potentially infected. Our reflex testing analysis indicates that in approximately 11% of pool deconvolution attempts, the positive PCR result was not confirmed, in turn making it impossible to exclude a false-positive pooled PCR result (which, in turn, would have a clear negative impact on all individuals in that positive pool and their families). Given that the pooled testing and reflex testing were not performed at the same time and given that the reflex test (BinaxNOW) is analytically less sensitive than the PCR (8), it was not possible to formally determine a positive predictive value for the pooled PCR. However, a separate analysis of data for the few schools in the program using individual PCR (rather than BinaxNOW) for positive pool deconvolution indicated a similar percentage of unconfirmed PCR results (9%), also limited to pooled samples with high C_T values. It was also not possible to calculate a negative predictive value for the pooled PCR, as individuals in pools with negative results did not receive any additional testing. Additional challenges worthy of consideration for program implementation include courier logistics, result turnaround time, and management of private health information in the school setting.

Decisions about school screening for SARS-CoV-2 infection are complex and must take into account both operational and analytical program performance data (as evaluated in this study) and evidence for or against program efficacy and cost-effectiveness that future studies will address. The true added benefit of regular SARS-CoV2 screening in the school setting, when added to other mitigation measures, still remains to be defined and may hinge not only on case prevention but also on the reassurance needed to keep schools open during a pandemic (12)—reassurance that is provided by the low school case rates observed in this pilot study. The Massachusetts DESE/DPH public K–12 pooled testing program has provided some of the first real-world data to help answer these critical questions and facilitate the development of policies and best practice for fall 2021 and beyond.

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