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Saliva-Based, COVID-19 RT-PCR Pooled Screening Strategy to Keep Schools Open

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Abstract

Background: As of March 2020, governments throughout the world implemented business closures, work from home policies, and school closures due to exponential increase of coronavirus disease 2019 (COVID-19) cases, leaving only essential workers being able to work on site. For most of the children and adolescent school closures during the first lockdown had significant physical and psychosocial consequences. Here, we describe a comprehensive Return to School program based on a behavior safety protocol combined with the use of saliva-based reverse transcriptase-polymerase chain reaction (RT-PCR) pooled screening technique to keep schools opened.
Methods: The program had 2 phases: before school (safety and preparation protocols) and once at school (disease control program: saliva-based RT-PCR pooled screening protocol and contact tracing). Pooling: Aliquots of saliva from 24 individuals were pooled and 1 RT-PCR test was performed. If positive, the initial 24-pool was then retested (12 pools of 2). Individual RT-PCR tests from saliva samples from positive pools of 2 were performed to get an individual diagnosis.
Results: From August 31 until December 20, 2020 (16-wk period) a total of 3 pools, and subsequent 3 individual diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease were reported (2 teachers and 1 staff).
Conclusion: Until COVID-19 vaccine can be administered broadly to all-age children, saliva-based RT-PCR pooling testing is the missing piece we were searching for to keep schools opened.

As of March 2020, governments throughout the world implemented business closures, work from home policies, and school closures due to rapid increasing rates of coronavirus disease 2019 (COVID-19) cases, leaving only essential workers being able to work on site.[1] For most of the children and adolescents, school closures during the lockdown not only severely disrupted their education, but also had significant physical (aberrant dietary and sleeping habits, hunger from missing free school meals in deprived areas), psychological (monotony, distress impatience, annoyance, neuropsychiatric manifestation), and psychosocial (domestic violence, child abuse) consequences.[2,3] In addition, the school lockdown was associated with a significant increase in poverty, as the school closure prevented many parents from attending work due to daytime caring responsibilities.[4]

Parents, pediatricians, psychologists, social workers, hospital authorities, government, and nongovernmental organizations have an important role in mitigating the psychosocial ill-effects of COVID-19 on children and adolescents. The development of innovative tactics that include affordable testing models to achieve acceptable risk levels is imperative to keep schools opened in the "new norm" era of COVID-19.[5]

The reopening of schools has significantly increased the student's risk for contracting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). High levels of SARS-CoV-2 shedding in the upper respiratory tract can lead to aerosol transmission of SARS-CoV-2, highlighting the risk that schools/universities take as students gather in close locations during several hours per day.[6,7] Moreover, up to 40% to 45% of people infected with SARS-CoV-2 may never be symptomatic and, therefore, not detected by clinical screening.[8] However, viral spread from people without symptoms may account for more than 50% of transmission events in COVID-19 outbreaks.[9] Therefore, the only way to reopen schools is to develop strategies to rapidly detect asymptomatic infected individuals and trace and isolate suspected COVID-19 cases and their contacts.[10,11] Principals are now faced with a plethora of ad hoc available testing options that takes into account their specific needs with regard to incidence of the disease in their states, and the unique characteristics of their schools/colleges.[12–14]

To date, the gold standard test to diagnose SARs-CoV-2 infection is the real-time reverse transcriptase-polymerase chain reaction (RT-PCR).[15] The initial sample collection method

involved the use of a nasopharyngeal (NP) swab, that causes patient discomfort and requires medical supervision. Recently, NP swabs has been substituted by saliva collection, which does not require specialized consumables, causes less patient discomfort, and can be self-collected (no medical supervision required), reducing the risk of transmission and decreasing the cost.[16–27] The effectiveness of this approach has been confirmed through validation studies.[28] However, individual, diagnostic testing strategies, applied to all the population, are too expensive. For this reason, pooling strategies, initially with NP swabs and later on with saliva samples, have been developed to screen large populations to detect asymptomatic infected individuals.[27,28] Pool testing can identify and isolate asymptomatic carriers, thus enhancing the health and safety of those attending school/work. A recent study by Watkins et al., suggested the use of large screening pools when the case prevalence rate is <3% and smaller pools when incidence is >3%.[28]

Here, we describe a comprehensive Return to School program based on a behavior safety protocol combined with the use of pooling of saliva samples as screening technique, conducted between August and December 2020 in a K-12 independent school in New York. The model included pre-entry student education and training, as well as symptom and exposure tracking. Once at school, a screening program, based on weekly RT-PCR on pools of saliva samples of all students and faculty/staff was used to identify the infected, albeit asymptomatic, individuals.

Methods

Study Design

The "Safe Return to School Program" was tested at Hackley School located in Tarrytown, Westchester County, New York. As of December 24, 2020, the percentage of individuals who tested positive in this county was 5.7%.[29] In addition, the global number of confirmed cases and death was 65,137 and 1628, respectively.[30]

Hackley School

Students

Hackley School is a K-12 independent school with 840 students coming from over 100 zip codes throughout the tristate area surrounding New York City. The school is located on a 285-acre campus, and has a small 5-d boarding population.

Educational Options

During 2020, Hackley School offered 3 different models to deliver its educational mission: (a) in-person - students attend classes being taught in-person and on campus; (b) hybrid - some students attend classes taught in-person using video conferencing, while others are present in the classroom; (c) distance learning - 100% use of technology platforms to advance the curriculum (this option would only be used if/when the school is forced to close, either due to state mandate or an outbreak on campus).

Safe Return to School Program

Before Going to School

1. *Safety Program:* It includes detailed information regarding Policies and Procedures (Annex 1) with regard to: protective personal equipment, physical distancing, hygiene and cleaning, visitor policy, travel to/from school, boarding program, upgrading of air systems (to improve air filtration) in the buildings, hand hygiene (touchless hand sanitary stations), transparent barriers (physical barriers in classrooms and offices, helping provide an extra layer of protection in high traffic areas and spaces where 6-feet of social distancing may be challenging), as well as signage regarding physical distancing, hand washing, masks, modified food service, transportation guidelines, and other important measures posted around campus, providing individuals a visual reminder of the precautions required. Finally, families were required to acknowledge/sign a community pledge relating to risk behaviors for COVID-19 transmission (Annex 2).

2. *Informational Program*: To educate and train students/faculty/staff/families about the disease during pre-entry and post-entry, the school developed a COVID-19 Frequent Answer and Questions annex in their webpage (Annex 1) that contains leading up-to-date, scientific, medical, and consumer relevant information about SARS-CoV-2 epidemiology, screening and surveillance protocols, test interpretation, as well as Centers for Disease Control and Prevention (CDC) and government safety protocols.[13,14] In addition, the school organized 4 reopening zoom calls with families and employees to answer their questions and to address all their concerns about the return to school.

3. *Preparation Program*: It included symptom and disease exposure tracking. Before returning to work, all employees will register and complete a daily Symptom Tracker and contact tracing survey (Hackley School Employee and Visitor COVID-19 Health Screening Questionnaire (Annex 3 and 4) to classify students/faculty/staff in 3 groups:

This survey classified students/faculty/staff into 2 groups:

1. *Those who can attend school*: If the student/faculty/staff or visitor has no fever and answered "No" to all questions, the individual can enter campus and attend in person or hybrid-model.
2. *Those who cannot attend school*: If the student/faculty/staff or visitor had a temperature greater or equal to 100.0°F or if the individual answered "Yes" to any of the screening questionnaire questions, they were advised to contact their primary healthcare provider, follow CDC guidelines, and attend distance learning (on-line) classes.

Once at School

A combination of the Safety (previously described) and a Disease Control Program took place.

1. Disease Control Program: It included the saliva-based RT-PCR pooled testing protocol and contact tracing. Before the students/faculty/staff participation in the screening protocol, they all had to signed an informed consent (Annex 5). This form gave Hackley School permission to test the student's samples and release the results to the school. All students, whether participating in in-person instruction or hybrid learning instruction to start the year had to complete this form. Participation in pool testing was required for students to be on campus for any in-person classes or activities:

a. Sample Collection

At the beginning of every month, every student received 4 individual collecting kits that contained: saliva collection tube; saliva straw; alcohol wipe, tube bag, and instructions for collecting the sample (Figure 1). The first collection took place on campus under the supervision of trained medical professionals and subsequent samples were collected at home and then checked in on campus. Sample check-in involved a no-contact technique (scanning registration card and sample only). The school ensured that samples

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Components: Saliva straw (a); Saliva collection tube (b); Alcohol wipe (c), tube bag (d) and instructions for collecting the sample (e).

Figure 1. Individual sample collection kit.

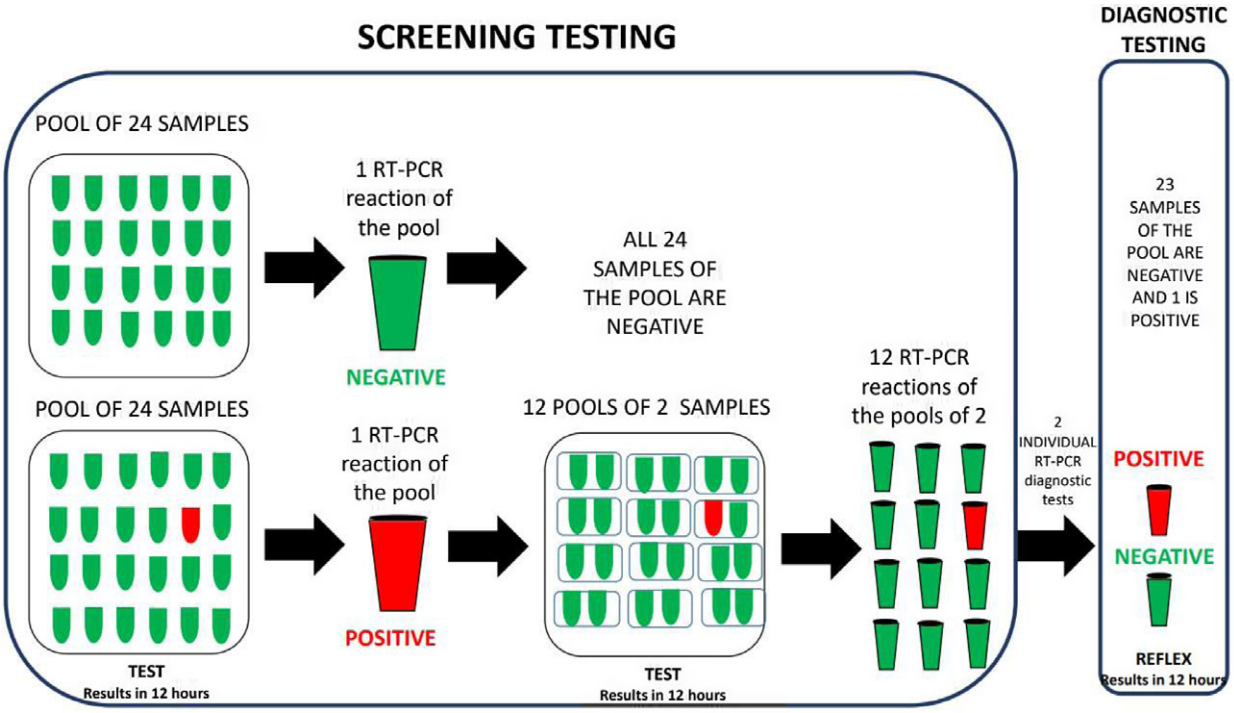


Figure 2. Saliva-based RT-PCR pooling test: Flow chart.

and individuals were correctly matched. This information was not shared with any third parties.

b. Schedule for Sample Collection

Hackley school performed a weekly routine of testing of all students, employees, part-time coaches (while in-season). Every Friday, students provided a saliva sample during 1 of the following time windows: 7:30-10:30 AM and 4:00-5:00 PM on Thursdays for the second-shift janitorial crew. To help keep the flow of people on campus more manageable, the students were requested to bring the saliva samples in 1-h windows for each level (lower, middle, upper school, and overflow). Samples from the lower school (K-5) were collected in their homeroom by 2 nurses. Based on the end-of-week testing schedule, individuals tested received the results by Monday morning, before going to school, as to whether they can return to campus.

c. Saliva-Based RT-PCR Pooling Testing

c1. Pooling Sample Process

Figure 2 summarizes the pooling sample process. Each saliva sample was assigned a barcode; therefore, no personal information was shared with the lab conducting the pooling tests as they only receive barcoded samples. Saliva samples from 24 individuals were pooled in 1 test to reduce the number of tests, and an RT-PCR was performed.[31,32] If 1 person within a pool of 24 saliva samples (POOL TEST) tested positive, the entire pool was flagged as positive and subsequent testing (RETEST, 12 pools of 2) was enacted to discern which individual(s) within the pool was/were the case/s. When a pool of 2 tests positive, the 2 individuals in that pool were contacted for consent to do an individual diagnostic PCR saliva-based test (REFLEX TEST).[31,32] This test was performed with the existing saliva sample. Results were

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Table 1. Types of COVID-19 tests

Type of test	Characteristics
Screening Test	It is intended to identify infected individuals prior to development of symptoms or those infected individuals without signs or symptoms who may be contagious, so that measures can be taken to prevent those individuals from infecting others. It looks for occurrence at the individual level even if there is no individual reason to suspect infection such as a known exposure.
Diagnostic test	It is intended to diagnose an infection in patients suspected of COVID-19 by their healthcare provider such as in symptomatic individuals, individuals who have had a recent exposure, individuals who are in a high-risk group such as healthcare providers with known exposure, or testing to determine resolution of infection. Diagnostic tests may also be appropriate in areas of high community spread, at the discretion of the ordering healthcare provider.
Accuracy:	
Pooling test	*Sensitivity:* Pool sizes of 5, 10, and 20 lead to detection sensitivities of 92.59% (95% CI: 88.89, 95.56), 88.89% (95% CI: 80.00, 91.85), and 85.19% (95% CI: 75.56, 91.11) of samples relative to that of unpooled samples[28]. *Specificity:* The test detects three different unique viral RNA templates. For a positive result, at least two RNA templates must be detected. The protocols used substantially decrease false positives created by detecting dead viral particles[32].
Individual test	*Sensitivity*[28]: 100% *Specificity*[28]: 100%

provided within a couple of hours from the consent. For those couple of hours, the individuals were asked to isolate. Table 1 summarizes the differences between screening and diagnostic COVID-19 tests as well as the accuracy of individual versus pooling COVID-19 tests.

c2. Laboratory Testing Platform

Every saliva collection tube was assigned a barcode (de-identified sample). No personal health information (PHI) was shared with the laboratory conducting the tests as they received barcoded samples (Mirimus Inc., Brooklyn, NY). The school ensured that RT-PCR tests from de-identified saliva samples and individuals were correctly matched using a third-party provider. After collection, all samples were shipped to the testing laboratory and arrived the same day. The saliva samples were used for COVID-19 testing only, and only approved school administrators were allowed access to all pool results. When a definitive diagnostic test was required a distinct Health Insurance Portability and Accountability Act (HIPAA) compliant system (Meenta Inc, Boston, MA) was used. The Meenta platform provided the physician's order, and the results were available to individuals and to the school.[33] The physician who placed the order attempted 3 calls to the phone number that was provided to explain the diagnostic test results and answer any questions. If the individual preferred not to be tested for diagnosis with the laboratory, the students were asked to arrange for a PCR test through their primary care physician.

c3. Contact Tracing: If the diagnostic test results confirmed the student was positive, the results were reported through a secure, HIPAA compliant reporting platform. The student/faculty/staff began self-isolation and received a call from an appointed contact tracer. Individuals who tested positive had to quarantine or isolate, depending on the presence or absence of symptoms, until meeting clinical clearance criteria in accordance with CDC guidelines and being clinically cleared by a qualified provider.[34]

Results

Students, Faculty, and Staff Demographics

A total of 840 K to 12 students ($n = 205$ from kindergarten to grade 4; $n = 240$ from grades 5 to 8; $n = 395$ from grades 9 to 12), 123 teachers, 31 administrators, and 49 staff ($n = 203$ total employees) are currently participating in program. The ratio of student/faculty is 7:1, 39% students are of color, with a 16 average class size. With regard to faculty, 36% are full time faculty in school housing, and 16% are faculty of color.

A total of 30 students participated in the 5-d Boarding Program.

Duration of the Safe Return to School

The Safe Return to School program was initiated August 28. The data contained in this report covered until December 2020 (16th week period).

Saliva-Based RT-PCR Pooling Tests: Results

A total of 43 pools of 24 individuals were run weekly, for a total of 516 pools so far (during the first week and the 16th week-period of screening, respectively). Of those, 3 pools of 24 individuals turned with a positive result and a re-test in 12 pools of 2 took place. Of those, 3 pools of 2 turned out to be positive. Globally, a total of 6 individual diagnostic tests were performed.

Positive, Individual Diagnostic Results

Once the individual diagnosis was performed, 3 positive individuals were reported. The first 2 cases corresponded to 2 teachers, before school initiation, who were quarantined and their contacts identified. The third positive took place the 11th week, limited to a nonteaching employee who was identified an isolated. A small cohort of other nonteaching employees was quarantined. None of this forced school closure or led to quarantine students or teachers.

Discussion

There is an obvious interest in keeping schools, universities and other educational facilities opened for physical attendance by students. As previous studies conducted during the first wave of the pandemic have shown, the impact on the children, adolescents and college students, with regard to intellectual, physical, psychological and psychosocial intellectual development, has been enormous.[2,3] Not only on the kids, but also on the parents who, in many cases, have witnessed the inability to keep their jobs due to the need to implement daycare activities with their children during the lockdown.[4]

However, it is necessary to do it safely. Recommended safety measurements like social distancing, masking and washing hands

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are effective in decreasing viral spread. In addition, screening for symptoms and contact with positive individuals prevent students with high probability of infection from attending school. Despite, it is well-known that up to half of infected persons remain asymptomatic while still spreading the virus.[8] In a recent screening study conducted among cancer patients in a hospital in NYC, the prevalence of viral detection was 4.23 % (95 % CI 2.05-7.65) (unpublished data). These asymptomatic cases can only be detected by diagnostic RT-PCR viral testing. While RT-PCR for SARS-Cov2 remains the goal standard, individual diagnostic testing at frequent intervals in large groups is not feasible due to the high cost associated. Rapid antigen test is a reasonable alternative to RT-PCR in terms of cost, albeit its low sensitivity compared with RT-PCR prevents its use for initial COVID-19 diagnosis.[35] Another option that could be considered is the use of RT-PCR based pooling strategies.

Here, we provide our experience with saliva-based, RT-PCR pooled screening strategy for school setting. The results show that this approach allowed the identification and isolation of infected students and maintained the school safely operational. As shown in 2 recent reports, children should not be considered super-spreaders of the disease, but, rather, also observed in other surveillance studies conducted in other schools (unpublished data), similar or even lower spreaders, as shown in the present study, compared with adults.[36,37] As stated by Levison et al., the safest way to open schools fully, and more importantly, to keep them open, is to reduce or eliminate community transmission while ramping up testing and surveillance.[38] The present study shows the feasibility of conducting test screening studies on children attending schools to detect, in a very rapid manner, those asymptomatic cases that are invisible to the symptom screening, mostly used in these settings. The saliva-based pooled testing strategy has been shown to be feasible (saliva samples required, sample collection process easy to implement) and affordable (the cost of the pooling testing is 6 times lower compared with individual diagnostic tests). More importantly, the use of the pooling technique decreases the demand needs as: (1) no swabs are needed to collect the samples; (2) only 1 RT-PCR test is run for every 24 samples in the pool, with the subsequent savings in reagents and materials.

Another important point to highlight is the good response of the parents to the community pledge relating to risk behaviors for COVID-19 transmission. This corroborates the fact that, to prevent the spread of the virus the establishment of strict, but easy to follow, safety protocol, that includes the 3 most important ways to prevent disease transmission (wearing mask, keep social distancing and wash hands), is the best way to ameliorate the spread of the disease until the percentage of people fully vaccinated increases—it is currently 56% including all U.S. regions.[39–42]

Conclusions

The data presented here are strongly suggestive of the ability to safely, effectively, and affordably keep schools open with the use of good infectious prevention behaviors, public health screening tools, and a saliva-based RT-PCR pooling testing strategy. It can be concluded that, until the COVID-19 vaccine can be administered broadly to all-age children (currently available only for those ≥12 y of age[43]), saliva-based RT-PCR pooling testing is the missing piece we were searching for to keep schools opened.[44]

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/dmp.2021.337

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