Considerations for Pooled Testing of Employees for SARS-CoV-2

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Objectives: To identify important background information on pooled tested of employees that employers workers, and health authorities should consider. **Methods:** This paper is a commentary based on the review by the authors of pertinent literature generally from preprints in medrixiv.org prior to August 2020. **Results/Conclusions:** Pooled testing may be particularly useful to employers in communities with low prevalence of COVID-19. It can be used to reduce the number of tests and associated financial costs. For effective and efficient pooled testing employers should consider it as part of a broader, more comprehensive workplace COVID-19 prevention and control program. Pooled testing of asymptomatic employees can prevent transmission of SARS-CoV-2 and help assure employers and customers that employees are not infectious.

Learning Objectives

- Discuss the potential advantages of pooled testing of employees for SARS-CoV-2.
- Summarize the findings of the new review of evidence on pooled testing for SARS-CoV-2 in occupational settings.
- Discuss the implications for workplace strategies to prevent COVID-19, and to assure employees and customers that staff members are not infectious.

The control of SARS-CoV-2 in workplaces is critical to the health of the population and the national economy. Key strategies for controlling SARS-CoV-2, especially prior to the availability of an effective vaccine; include physical distancing, hand washing, wearing a mask, symptom screening, testing, contact tracing, isolation and quarantine, and workplace readiness. Focused testing of asymptomatic employees can prevent workplace transmission of SARS-CoV-2 and help assure employers and customers that employees are not infectious. Productive business enterprises necessitate healthy noninfectious employees who do not pose a risk of infection to other employees or the public. Assurances that employees are noninfectious may entail frequent ''screening testing''[1] as part of a larger prevention and control effort that includes symptom and temperature screening and other workplace readiness and control interventions.[2] Viral testing may be used to determine whether SARS-CoV-2 nucleic acid or antigen is present in respiratory specimens. Laboratories may analyze individual respiratory samples to detect SARS-CoV-2 infection and assist with diagnosis of COVID-19. However, employers who need or want to conduct viral testing of their employees may have limited access to, or resources for, individual testing of employees. Pooled testing is a tool that can be used to stretch laboratory and financial resources.[3–6] This paper provides background information for practitioners, employers, worker representatives, and public health authorities about pooled testing of employees for SARS-CoV-2.

Pooled testing (also known as ''group testing'' or ''batch testing'') is a process where a number of specimens are combined according to their specific type into one pooled specimen for a test that yields binary results (ie, positive or negative). A negative test result indicates that all individuals within the grouped specimens are negative. A positive test result indicates that at least one individual within the pool is positive, and re-testing of each specimen or subgroups of specimens is warranted to identify positive individuals.[6] Pooled testing can substantially reduce the number of tests needed to screen a population for active infection and the associated financial and opportunity costs for screening, especially when the prevalence of the disease in a workplace is low, which may be the case in many workplaces.[3,7] Successive rounds of pooled testing could be used to efficiently screen workers for active SARS-CoV-2 infection for purposes of transmission control[8] (Fig. 1). Detecting both symptomatic and asymptomatic SARS-CoV-2 infections is critical to controlling transmission in workforces and communities, and for evaluating the effectiveness of controls.

Pooled diagnostic testing has been applied to various infectious diseases such as HIV and Zika virus, and for surveillance and blood bank screening; additionally, the approach has been enhanced over the years.[6,9–17] With regard to SARS-CoV-2, there is a growing body of literature on the general utility of pooled testing (for nucleic acid amplification tests).[1,4,18-27] While there are various approaches for the molecular detection of SARS-CoV-2, most involve real-time reverse transcription polymerase chain reaction (rRT-PCR) assays. rRT-PCR assays allow the amplification and analysis of one or more molecular targets within the nucleic acid extract to be done simultaneously. Critical in assessing the results of pooled testing using RT-PCR assays are the amount viral titer of each specimen, the number of samples in the pool, and the prevalence of SARS-CoV-2 in the population.[28] Other assay methodologies, such as digital droplet PCR (ddPCR) and LAMP-Seq, may also be suitable in pooling situations.[29] LAMP-Seq, a barcoded Reverse-Transcription Loop-mediated isothermal AMPlification method, is highly scalable and could be used to analyze large numbers of specimens per day.[30]

Pooled testing may be particularly useful to employers in communities with a low prevalence of COVID-19, less than 10%,

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Clinical Significance: Pooled testing can substantially reduce the number of tests needed to screen a worker population for active infection with SARS-CoV-2 and the associated financial and opportunity costs for screening if the prevalence of the disease in a workplace is low.

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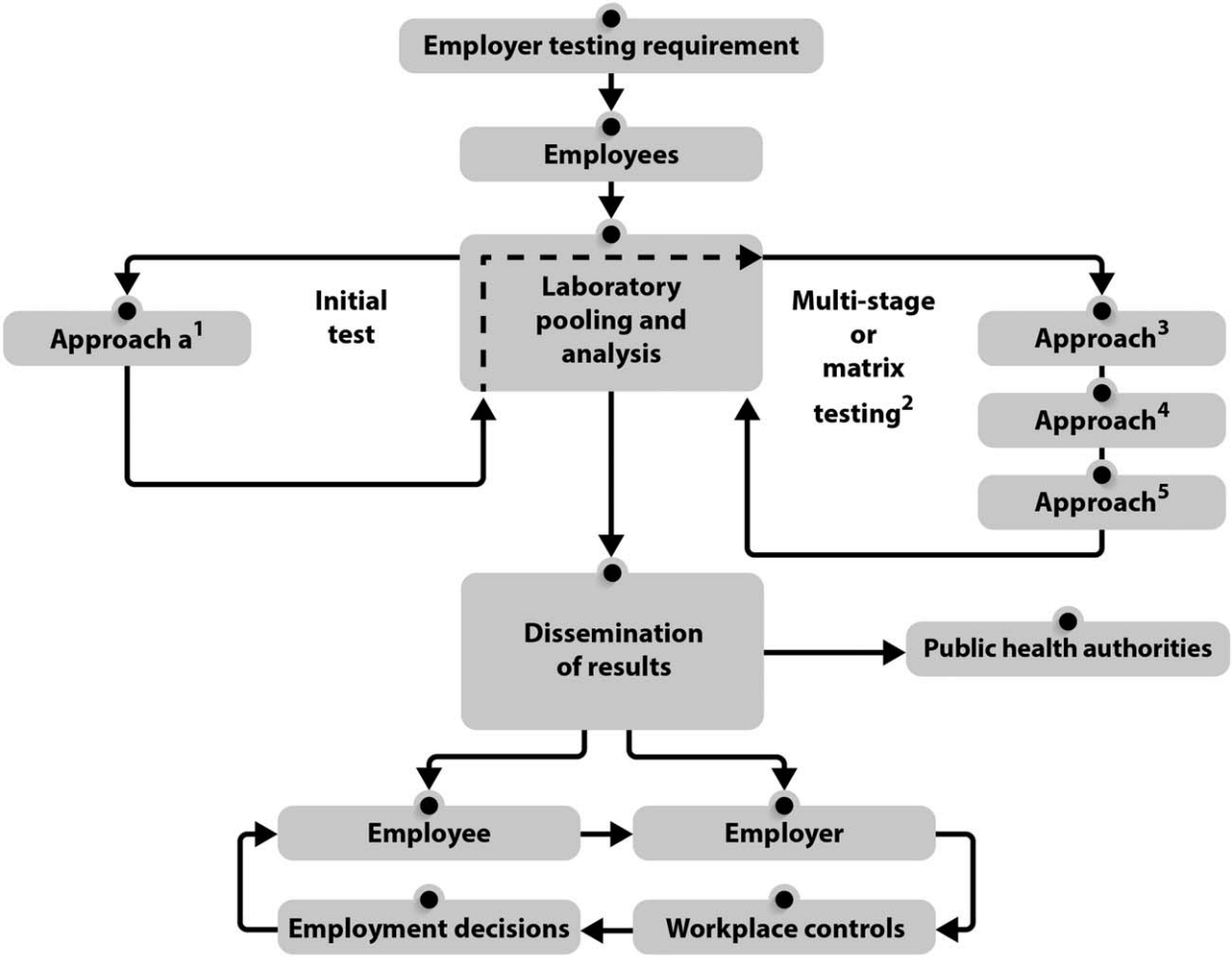

1: Specimens are mixed together in equal size groups and tested. If the group tests positive every specimen is retested individually (Mallapaty, 2020; Dorfman 1943).

2: Because of the limited information contained in a positive outcome, it is required to test certain specimens multiple, times – either in parallel for all specimens or sequentially with additional testing only for those specimen with positive results (Verdun et al 2020).

3: This approach adds extra rounds of testing to methods, reducing the total number of tests needed (Mallapaty, 2020).

4: This approach uses two rounds of testing. In the second round, specimens are tested in multiple overlapping groups, represented by rows and columns on a square. More people can be tested by arranging candidates on a cube (Mallapaty, 2020; Mutesa et al, 2020).

5: This approach uses one round of testing specimens are distributed into a matrix of overlapping groups (Mallapaty, 2020).

FIGURE 1. Process for pooled testing of employees.

and especially less than 2%.[7,24,31] Overall, pooled testing is more efficient when the prevalence is low.[32] The literature on the recommended number of samples in a pooled test is expanding for SARS-CoV-2, though most of that literature comprises preprints that have not been peer reviewed (https://www.medrxiv.org). Table 1 shows a representative sample of the literature on pooled testing for SARS-CoV-2. Early results from laboratories indicate that a single positive SARS-CoV-2 sample can be detected when pooled with many other samples of the same type. One study indicates that 64 samples can make up a pool that can be accurately analyzed.[25] Another indicates 30 samples is the better number depending on the gene assayed.[4] For larger pool sizes the specimen becomes more dilute. This limits the ability to detect borderline positive samples (ie, samples with viral load near the limit of detection) so maximum pool size must be determined by each laboratory under the conditions used for their RT-PCR analysis. Ultimately, the maximum pool size will vary by assay and laboratory.

For pooled testing to be effectively and efficiently carried out, employers should consider it as part of a broader workplace COVID-19 prevention and control program[1,2] that involves screening, testing, isolation and quarantine, contact tracing, and workplace readiness. CDC has extensive guidance on these topics.[1,2,62,63] Testing, in conjunction with other preventive efforts, is crucial for preventing transmission of SARS-CoV-2. Workplace readiness includes following the hierarchy of controls (engineering and

TABLE 1. Literature on Pooled Testing for SARS-CoV-2*

Investigator	Type of Study	Accuracy	Conclusion
Abid et al[5]	Test sensitivity of pools of various sizes	A single positive specimen can be detected in pools up to 10 with the same performance as standard RT-PCR.	Pooling would expand current capacity and be useful for hospital staffs and factory shifts.
Ben-Ami et al[19]	Demonstration	Pooling lysates retains clinical sensitivity	Pools of 5–8 significantly increase throughput
Bukhari et al[33]	Modeling	Study showed no significant effect of pooling negative specimens with positive in terms of detection of the positives by PCR	Application of algorithm to determine the appropriate number of specimens would be very cost effective
Cabrera et al[22]	Demonstration; Proposed methodology for pooled testing in care institutions,	Assumed sensitivity of 95% and specificity 100%. In order to minimize false negatives pools of 20 samples and sub pools of 5 samples were tested.	Proposed use of successive rounds of testing using a pooling approach for transmission control and to preserve testing resources
Cherif et al[34]	Simulation	Assessed pool size for different sensitivities. The false-negative rate may increase due to dilution of positive samples.	A probabilistic model can estimate the risk of false negatives based on COVID-19 prevalence, test sensitivity, and pool size.
Cleary et al[20]	Simulation of pooled testing validated by experimenting	Sensitivity decreases ''roughly linearly as the log of the dilution factor''	Group testing can ''substantially increase the identification rate of infected individuals in resource-limited setting''
Eberhart et al[35]	Simulation; multi-stage group testing	Referred to Yelin et al (2020) that pooling up to 16 samples could ''potentially not decrease test sensitivity.''	Group testing is more efficient than individual testing.
Eis-Hübinger et al[36]	Test pooling protocol in network of laboratories.	Ten-fold higher limit of detection	Laboratory-based minipools are easily adaptable and resource-saving
Escobar et al[37]	Modeling	Each two-fold dilution results in the increase of the C_t value by 1 unit on average	Using machine learning can increase pooling efficiency
Fang et al[38]	Simulation	False negative would be reduced to 2,000 from 35,000 for individual testing	Approach is seven times more efficient than individual testing
Farfan et al[39]	Demonstration	Reported lack of significant false negatives	Pooling nasopharyngeal samples proved reliable and thus a potentially efficient alternative to individual testing
Gan et al[40]	Validation	High viral load samples could be detected in pools with dilution folds ranging from 1/2 to 1/100; but low viral load detection was at very low dilutions	''Viral load significantly influences pooling efficiency.''
Ghosh et al[26]	Modeling	For 40–60 sample pools the method works with zero false negatives and zero false positives	Created a method to reconstruct viral loads of each sample with high sensitivity and specificity
Gollier and Gossner[41]	Simulation	Tests assumed to have no false positives or false negatives	For prevalence around 2%, pooled testing could reduce the number of tests by 95%.
Griesemer et al[42]	Tested different pool sizes using clinical specimens	Pool of 5: C_t values increased by 0.4—1.5%; weak positives detected	''Weak positive specimens were detected in all five-sample pools but failed to be detected in 4 of the 24 nine- sample pools tested.''
Guha et al[43]	Examine statistical theory behind pooled testing	Reduces misclassification among those tested positive	Theoretical results show that pooled testing is effective for reducing time and cost of screening
Gundersen et al[44]	Modeling	''. . .the relationship between ''pool size and test sensitivity is not yet fully established and might vary between individual laboratories''	Testing for SARS-CoV-2 using RT-PCR analyses can be substantially increased by using pooling techniques

TABLE 1. (*Continued*)

Investigator	Type of Study	Accuracy	Conclusion
Hanel and Thurner[45]	Simulation	Replicates should help lower false negatives. Estimates that at an infection level of 0.1% about one case in 800 (0.13%) will be missed. At 1%, one case in every 241 pooled tests (0.41%) will be missed.	The optimal pool size and efficiency of pooling strongly depends on the infection level of the population. "For infection level of 1% the optimal pool size is 11."
Heidarzadeh and Naryanan[46]	Modeling	"10 infected people in a group of 961 can be identified with 70.86 tests with an average sensitivity of 99.5% and specificity of 99.6%"	The proposed approach provides 13.5 times the throughput than with individual testing
Hirotsu et al[47]	Validation of pooling	Pooling of 20 specimens decreases PCR sensitivity (1.3 $\log_{10}$) (C_t increase 4.3))	Showed utility of screening healthcare workers with pooled testing
Hogan et al[18]	Retrospective	1 False positive in 292 pools	Pooled screening may facilitate detection of early transmission of SARS-CoV-2 and enable timely implementation of control measures
Lohse et al[4]	Demonstration	Compared cycle threshold for pools that tested positive with C_t values of individuals that tested positive	Tested 1,191 samples, 23 of which were positive; used pool size of 30 subdivided into pools of 10; need 267 tests. Pooling can increase capacity
Millioni[48]	Simulation	Compared sequential pooling with standard approach; as the fraction of true positives that are correctly assigned to pools increases so does the pool size.	Sequential pooling is more efficient than one-step pooling. With virus frequency below 5%, pools of 20–25 are useful.
Mulu et al[25]	Test clinical samples	Slight loss of sensitivity for sample pooling; no loss for RNA pooling	Proposed pools of four for direct biological samples and pools of eight for RNA
Mutesa et al[21]	Assessment of grouping subsamples prior to testing.	Positive specimens can still be detected after 100-fold dilution.	Costs of mass testing could be reduced by a factor often to a hundred or more.
Mutzel et al[49]	Simulation	With a 128 size pool samples with C_t value of 31 or above might escape detection	Use of a recursive method will result in reducing number of tests compared with individual testing by 83.5%. Pools should be no larger than 30.
Noriega and Samore[50]	Simulation	An "0.9 sensitivity loss leads to a relatively low increase in posterior probability of a disease after a negative test outcome"	"A pooled testing strategy has the potential to enhance comprehensive surveillance of SARS-CoV-2" when prevalence is low such as 3%"
Pikovski and Bentele[51]	Simulation	The specificity of a pooled test is increased compared to an individual test.	Pooling of tests increases the capacity for COVID-19 testing. A pool size of four is recommended
Pilcher et al[7]	Modeling	Approach increased the number of true positives and positive predictive value compared with individual testing.	"For a fixed number of tests group testing could screen 2–20 times compared to individual testing."
Shani-Narkiss et al[52]	Simulation	If viral DNA is diluted by a factor of eight from the original concentration in a sample it will take three cycles of doubling in order to reach the original concentration	In a low prevalence area, one-time pooling strategy with optimized initial batch size, is very efficient for prevalence up to 20%
Sharma et al[53]	Modeling	Multiple studies have confirmed that a pooling size of up to eight does not harm the sensitivity or specificity of the test	A mathematical model for pooling was developed and validated. While pooling for prevalence of less than 5% can use a pool size of 5. Pooling is not warranted for prevalence greater than 20%

TABLE 1. (*Continued*)

Investigator	Type of Study	Accuracy	Conclusion
Shental et al[54]	Method development proof-of-concept	"Simulations demonstrate that the method can correctly identify up to 5/384 (1.3%) of carriers, with an average number of false positives that was less than 2.75, and an average number of false negatives less than 0.33."	This pooling method produces an "efficient easy-to-implement approach for increasing testing capacity."
Sinnott-Armstrong[24]	Simulation	"Testing efficiency can"... be increased at the expense of specificity by pooling people in close contact (eg family group, work units into a single sample prior to group allocation or RNA extraction... individuals in close contact have positive correlated infection status... cluster identification" is more important than an individual's status.	"Testing mildly symptomatic and asymptomatic individuals using a group testing approach will discover the same number of cases as individual testing using same number or fewer tests" than individual testing." "When most tests are negative pooling reduces the total number of tests up to four-fold at a 2% prevalence and up to 8-fold at 0.5% prevalence."
Skorniakov et al[55]	Modeling	Tests applied perform equally well for individual and for pooled samples	The greatest gain in efficiency correspond to the lowest prevalence
Szapudi[56]	Model development	Accounting for false positives and false negative can be accomplished by using a likelihood function in a forward Bayesian analysis	Demonstrated that sample pooling is efficient as long as the fraction of population infected is relatively small.
Täufer[57]	Simulation	Provides error bounds on the number of false positives which scale favorable with large numbers and all be small in realistic situations	Non-adaptive pooling strategy allows for rapid and large-scale screening
Theagarajan[23]	Modeling	False negative rates or the sensitivity is an appropriated metric to evaluate group tests	Group testing is a promising method to increase the number of tests in COVID-19 diagnosis
Verdun et al[58]	Simulation	For a PCR sensitivity of 99% the reduction caused by pooling was 1%–2%.	Using an appropriate testing procedure can result in an up to 10-fold increase of the feasible throughput
Verwilt et al[8]	Simulation	Pooling methods suffer from false negatives to a variable degree	"Choice of pooling method and pool size involves a prevalence-dependent efficiency-sensitivity trade-off"
Viehweger et al[59]	Test sample replicates	"20-fold dilution, that is, pooling 20 samples would cause C_T value to increase by 4.3 cycles" which is still well above the detection limits.	"At 2% prevalence and 20 samples per pool the protocol increases screening capacity by factors of five and two compared to individual testing and traditional pooling, respectively."
Wacharapluesudee et al[60]	Test 28 ten-specimen pools	The sensitivity of viral RNA detection for each pool was compared with the sensitivity of PCR. No significant differences were found.	Pooling" can dramatically decrease resource burden on laboratory operations by up to 80%"
Yelin et al[25]	Pooling clinical samples	False negative rate: 10%; detected a single positive sample in pool of 32 samples	Pooling is useful for essential monitoring of work groups such as hospital staffs
Žilinskas et al[61]	Simulation	Assumes from literature that a single positive sample in pools of up to 32 samples can be detected with 10% false negative rate	Sequential pooling is more efficient than one-step pooling with prevalence below 5%; pools of 12–27 are useful.

PCR, polymerase chain reaction.
*Based on an inexhaustive search of the Yale medRxiv data base using the terms "pooled testing for coronavirus."

administrative) that involve ventilation optimization, physical distancing, use of structural barriers, hand hygiene, cleaning and disinfecting surfaces, cloth masks, surgical masks, and respirators.[2] The employer has the responsibility for providing a safe and healthy workplace. Having infected employees present in the workplace constitutes a hazard for other employees, customers and others. Consequently, the employer is obligated to minimize exposure of employees to other infected employees as described in existing Occupational Safety and Health Standards (OSHA) and the General Duty clause of the Occupational Safety and Health Act (https://www.osha.gov/SLTC/C0vid-19/). An employer may use viral testing to determine if an employee has COVID-19 as a condition of entering a workplace.[64] Testing individuals after resolution of symptoms is less likely to detect replication-competent virus and therefore not recommended for determining when an infected employee may return to the workplace.[65]

Performance of a viral test, analyzed individually or collectively in a pool, involves the collection of personal health information. Consequently, informed consent from employees is required prior to reporting results to the employer[27] 45 CFR 493.1291(1) 2019; 45 CFR 164.524 (e) (31 (ii) 2019). In addition, the Coronavirus Aid, Relief, and Economic Security (CARES) Act requires every COVID-19 testing site to report results to the appropriate state, tribal, local, or territorial (STLT) health department. Details on reporting and maintaining the privacy of results are available.[1] The approach to such reporting should be developed in collaboration with the laboratory analyzing the sample specimens and with state and local public health entities. Pooled specimens with positive results must be deconvoluted and discrete specimens retested individually before reporting individual results consistent with the Clinical Laboratory Improvement Amendments (CLIA). Collecting and saving duplicate samples from individuals making up the pool can prevent the need for going back and re-sampling workers. Employees must be notified of the results and isolated, if appropriate. Cases of COVID-19 identified through testing should be investigated, and their contacts should be traced and quarantined in collaboration with the appropriate STLT health department. Follow-up pooled testing of a workforce when the positive cases are removed from consideration due to isolation is also warranted to detect onset of new cases.[1,2]

DEFINING GROUPS TO BE TESTED

The groups of workers whose tests would be pooled should be defined based on the number and distribution of the workers in each workplace, the density of the workforce in each work area, and the types of tasks and interactions that could occur. There is a need to determine the size of and inclusion criteria for each group to be tested. For equity and legal reasons, the determination of workers who will receive pooled testing must be job-related and based on business necessity.[64] Defining the groups of employees to be tested should be done by someone knowledgeable in pooled testing design and in consultation with the laboratory performing the testing, since many logistical issues based on laboratory capacity will require consideration. It may be advantageous to test workers in ''pods'' (similar exposure groups) since employers may try to keep workers in stable teams (''pods'').[41] If infection occurs in the pod, spread would be contained to the members of that pod.

COLLECTING AND TRANSPORTING SPECIMENS

The procedures for collecting nasopharyngeal, oropharyngeal, or mid-nasal turbinate specimens for pooled testing have been described.[4,24,25,66] A challenge may be how the specimens get collected from workers in a workplace for pooling in the laboratory. The need for testing and the use of a pooled testing approach should be included in an employer's communication to employees on how COVID-19 will be addressed in each workplace. This should include a description of the pooled testing process. Employees should also be informed of where and how specimen collection will occur, the frequency of collection, and the handling of test results. Since a diagnostic test for SARS-CoV-2 is considered personal health information, employees may have to sign a release or consent form to allow the results to be shared with the employer or the employer's occupational health provider. If the enterprise has a medical facility, or designated occupational medicine provider, that could be the location for the specimen collection and pooling. If there is no enterprise-associated medical facility or provider, the means of collecting and pooling specimens may be more difficult and will need further consideration. Any specimen collection at the workplace should be conducted in a private area to protect the workers' privacy. Procedures should be in place to protect the confidentiality of information involved in specimen collection and with test results. Measures should be taken to ensure safe collection and handling of specimens.[67,68] All specimen mixing and pooling should be done by trained laboratory personnel and be consistent with FDA guidance for the assay used.

Collection and handling of specimens may present safety and health hazards and manual mixing may increase the potential for operator exposure to SARS-CoV-2.[69] Therefore, strict biosafety precautions should be taken.[67,68]

ACCURACY OF POOLED TESTING

The utility of pooled testing to identify individuals infected with SARS-Cov-2 has been demonstrated.[20-22,25,31,33,42,48,60] Because samples are diluted when pooled, proportionally less viral genetic material is available for detection, resulting in a greater likelihood of false negatives,[38] which can vary by method.[8] However, a number of studies indicate that a weakly positive sample within the limit of detection of the assay can be identified in pooled studies.[4,7,19,25,31,38,60,70] Currently, the limited literature indicates that, with a pool the size of 32, the sensitivity is 90% relative to that of a single analysis (10% false negative rate); ''sensitivity here refers to sensitivity of the pool versus sensitivity of testing each of the individuals in the pool'' and pertains to one study and one model.[25,44]

When considering the accuracy of pooled testing, there is a tradeoff between efficiency (the total number of samples tested divided by the total number of tests performed) and sensitivity.[20] The sensitivity of a test for SARS-Cov-2 is a function of many factors, but of particular importance, is the within-host viral kinetics, because the viral load within a person can vary by at least six orders of magnitude over time.[20,59,71,72] Tests on positive SARS-CoV-2 specimens diluted up to 100-fold show that the virus can still be detected.[21] ''The number of samples that can be pooled without affecting the PCR sensitivity is limited by the C_t for the target, that is, the cycle at which amplification becomes detectable over background noise''.[73] Testing groups with low prevalence of SARS-CoV-2 could reduce the number of false negatives and false positives compared with individual testing.[13,74] Moreover, ''the optimal test design and group size ultimately has to be estimated taking resource constraints, pre-test probabilities, expected number of tests, and expected false positive and false negative rates into account''.[74] Also, the influence of prevalence on efficiency and sensitivity needs to be considered. In order to make an informed decision on pooling, prevalence needs to be known, but as this can only be estimated before testing[8]; estimates of local prevalence of SARS-CoV-2 should be obtained in collaboration with STLT health officials.[2,63] Laboratories may determine prevalence based on their own experience with SARS-CoV-2 testing by using the rolling average of positive tests over the previous 7–10 days.[1]

LABORATORY ANALYSIS OF POOLED SPECIMENS

When using a pooled testing procedure to generate results that are not specific to any one individual, the Food and Drug

Administration (FDA) does not require laboratories to have CLIA certification during this COVID-19 public health emergency. There are two FDA authorizations for use on pooled specimens (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-facilitating-diagnostic-test-availability-asymptomatic-testing-and). The first authorized test allows for testing up to four samples in a pool. The second authorized test uses a matrix approach that involves testing up to five samples per pool and 25 samples per matrix (https://www.fda.gov/media/136151/download). There may be a potential of obtaining false positive or false negative results when utilizing a pooled analysis approach.[61] Consequently, the Centers for Medicare and Medicaid (CMS) indicates that: ''Surveillance with pooled or batch testing should be validated on a test platform, and tests of high sensitivity and positive tests should have a confirmatory test.'' It is not clear if this refers to a confirmatory test on pooled specimens or on the need to retest individuals to identify the positive individual(s) within the pool. However, follow-up individual testing of subjects within a pool must be done by a CLIA certified laboratory because when patient-specific results are obtained, then a CLIA certificate is required.[74]

FOLLOWING UP OF POOLED POSITIVE TEST RESULTS

The literature on pooled testing includes various approaches for following up when a pooled test is positive. Much of the literature on pooled testing supports using an adaptive approach where selection of the optimal testing scheme is based on the expected prevalence rate.[35,48] Generally, when each specimen is collected, it is split into two aliquots: one for the pool and one for follow-up testing if necessary. Various combinatorial matrix arrangements for specimens in pools have been proposed. These are generally algorithms for determining efficient ways to test specimens following a positive test in a pooled specimen.[21] For example, the specimens for a workforce of 96 workers can be arrayed in 8 pools of 12 workers forming an 8×12 matrix that can be arranged sequentially from 1 to 96.[24,61] Then the 8 pools are tested (the rows) and then the 12 pools are tested (the columns). Where a positive row and column pool intersect that identifies an infected person. There may be more than one infected person among the 96 and that will increase the number of tests needed. With just one infected person among the 96 the number of tests needed will be 20 instead of 96. The pool size will depend on prevalence in the group and viral load in each worker. Laboratories performing or analyzing tests to detect SARS-CoV-2 or to diagnose COVID-19 are required to report the results to STLT public health departments (CARES Act Section 18115). Workers with positive tests will need to be informed of their result and given instructions for isolation. In workplaces, those who came in close contact with other workers who tested positive will need to be identified, informed, and advised to follow the CDC guidance for contacts.[62]

FREQUENCY OF TESTING

Pooled testing is a useful tool in workplaces in low-prevalence communities, that is, those communities with 10% or less prevalence of COVID-19. However, much lower prevalence, such as less than 5%, or 2%, is more advantageous for pooled testing.[24,32] The frequency of pooled testing will be a function of the community prevalence of COVID-19, the prevalence in a workplace, and the availability of individual testing. Repeated targeted pooled testing may have value because it allows employers to continually monitor a disease such as COVID-19 that spreads rapidly. Moreover, repeat testing helps identify cases that might have occurred since the last test or had been missed previously since PCR tests miss about 20%–30% of infected cases, whether pooled or not.[75,76] The most efficient frequency for repeat testing will vary by workplace and may change over time. While there is a relatively large amount of literature on optimal group testing strategies, there is little in the literature on the frequency for repeating pooled testing for SARS-CoV-2.[6,32,49,77,78] However, there is wide support for the concept of repeat pooled testing and its use in routine monitoring of populations such as workers.[5,21,22,31,48,54,56] Cabrera et al[22] concluded that after an institution achieved a prevalence of zero, and after exclusion of positive and symptomatic people, new cases identified by multiple rounds of screening using pooled testing would be presymptomatic or newly symptomatic individuals with viral loads just reaching their peak. In this situation, considering a doubling time higher than 14 days, test rounds of 14 days could be adequate to detect any new highly infectious person.[22] This timing could be adjusted depending on turnaround time of the laboratory, incidence of local virus transmission, prevalence of infection, risk severity and population tolerance of the sampling methods.[22] However, Larremore et al,[79] using simulation methods, found that weekly surveillance testing, when coupled with isolation of infected people would attenuate surges of infections, but that dramatic reductions of total infectiousness were observed by ''testing daily or every third day, $\sim$ 60% reduction when testing weekly and $<$40% under biweekly testing.'' In general, testing more frequently lowers the prevalence rate by containing infections.[32]

MANAGEMENT OF POOLED TESTING

Pooled testing likely will be initiated and managed by employers and delegated to onsite healthcare providers or at a contract laboratory. Pooled testing can be a complex effort to manage.[42,73] There are numerous technical hurdles to overcome.[24] The timing and coordination needed to pool specimens and track individuals requires planning and resources.[80] Without adequate and appropriate coordination, pooled testing can result in slow response time, which could impact the isolation of positive cases and quarantining of contacts.[26,42,73] However, when prevalence of infection is low, there will be a need for fewer follow-up tests and pooled testing can yield results relatively quickly. Key in the management of pooled testing is informing employees of pooled and individual test results, maintaining the confidentiality of the test results, then using the results to make decisions about whether the employee can work or will need isolation. Effective risk communication to tested employees is needed to prevent false reassurance after a negative pooled result.[50]

Whether or not individuals may be reinfected with SARS-CoV-2 is presently under investigation. If individuals may be reinfected with SARS-CoV-2, reinfection may have an impact on case surveillance and contact tracing and indicate the need to adjust prevention and control.[81]

Pooled testing in the workplace or under the auspices of the employer will present challenges. Its application is likely to expand when ease of use, flexibility, adaptability, cost efficiency, and rapid turnaround times are addressed. There are various software packages, apps, and algorithms developed in many countries that have been proposed to make the employer's selection of pool size relatively easy.[23,26,33,38,59]

UTILIZATION OF POOLED TESTING RESULTS

Primarily, the results of pooled testing will serve to identify employees who are infected and then can be isolated, their contacts traced, and those contacts, if non-employees, can seek testing or quarantine. Many contacts within the workplace most likely will be part of the testing pool but, if not included, a new pool could be developed to test them if there is known exposure. In addition, employers might utilize flexible sick leave and supportive policies and practices to encourage employees to not work if they feel ill.[2] Importantly, employers should constantly stress that negative test results are not a substitute for continuing effective safety and personal protection practices.

A BRIDGE TO THE NEXT GENERATION OF TESTING

Pooled testing, while potentially cost effective, can also be a bridge to the next generation of testing which includes point-of-care tests and other tests that can be used widely, frequently and quickly. It seems apparent that workforce functioning in the pandemic will ultimately require frequent testing.[79,82] Until that can be realized, pooled testing may help to extend resources and be a useful tool for employers and workers.

CONCLUSION

SARS-CoV-2 has been described as ''an ideal candidate for pooled testing'' because the viral load in persons increases quickly, plateaus for a while, then drops quickly; consequently, the window of detection is relatively long.[7] A range of specimen pooling protocols have been assessed.[3,7,18,19,25] Pooled testing has been shown to work in Wuhan, China, and in various clinical situations in the USA and elsewhere.[7,19,83] Pooled testing on saliva may be a useful approach that is promising but not widely investigated for use in diagnostics.[32,84,85] Limiting factors include availability of collection materials, reagents, and laboratory capacity to manage pooled testing, but the main driving factor for use of pooled testing of employees is using this process in workplaces with low prevalence (less than 10% but more appropriately less than 2%). The efficiency gained from pooled testing could help employers stretch testing resources and increase the number of employees who would be tested.

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