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Optimization of Multi Two-Level Batch Testing

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Abstract

As the COVID-19 pandemic continues to spread, it is paramount that the most efficient testing method be identified and implemented to reduce the strain on the medical community. This project introduces a novel batch testing method called multi two-level batch testing. Based on simulation, the multi two-level procedure is more efficient (at 89%) than both the two-level and the multi-level procedures at an infection rate of 1%, which is the anticipated rate at the University of Florida during the Spring 2021 semester. If laboratories implement multi two-level batch testing, they may reduce costs and labor. Additionally, this novel batch testing procedure can be applied to other diseases and future pandemics.

Keywords: batch testing, COVID-19, pandemic, simulation, Java, optimization, probability

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as COVID-19, is a virus which causes acute respiratory infection and typically afflicts the infected individual with a wide array of flu-like symptoms (Menni et al., 2020). Testing for COVID-19 has strained the medical community in terms of time, equipment, money, and labor, and testing must become more efficient to meet the increasingly large demand. This is especially true on college campuses, where communal housing, social events, and classroom instruction present unique circumstances that facilitate the spread of COVID-19 (Wilson et al., 2020). Many universities are implementing quarantine procedures and safety measures to protect students. For instance, the University of Florida is mandating that all students deemed at high risk for contracting the virus must test biweekly or forfeit their privileges of staying on campus and using campus resources (*Testing Plans for Spring Semester*, n.d.). With such a high volume of potentially infected individuals, means to decrease testing time and costs are desirable.

In 1943, Robert Dorfman published a seminal paper which detailed a method for more efficiently testing for syphilis in enlisted soldiers (Dorfman, 1943). He proposed two-level batch

testing, which involves the innovative strategy of pooling and testing multiple samples at once. If the result of the initial group test is negative, then every individual in that sample is presumed negative. However, if the initial test is positive, every member of the group is retested individually to identify those who are infected (Ahn et al., 2020; Dorfman, 1943). This two-level batch testing procedure has been modified in a new method called multi-level batch testing, which uses the same initial methodology as two-level testing, but subdivides each positive pool of samples into subgroups, and each subgroup is tested (Li, 2020). This study introduces and examines a new method called multi two-level batch testing, which is hypothesized to increase the efficiency of batch testing in terms of minimizing the number of tests run.

Methodology

The experimental portion of this research will constitute a simulation of various batch testing techniques: the two-level method (Dorfman, 1943), the multi-level method (Li, 2020), and the currently proposed multi two-level method. To systematically evaluate the efficacy of each batch testing method, a Java simulation was written to identify the minimum number of tests required for each method to determine the infection status of each individual in the population.

Two-Level Batch Testing

In the two-level test, visualized in **Figure 1**, individual samples are grouped into batches of size n (Dorfman, 1943). It is known that batches which test negative for the pathogen do not contain any infected members (Dorfman, 1943). However, batches which test positive necessarily contain between one and n infected member(s). Thus, all members of the positive-testing groups are individually retested in order to determine which specific members are infected (Dorfman, 1943). This method proves notably more efficient than testing each member individually when the percentage of the population infected with the pathogen $p \leq 0.30$; however, its efficiency is highly dependent on n, whose optimal value is in turn directly and exclusively dependent on p (Dorfman, 1943). For instance, when $p = 0.15$, there is on average a 28% reduction in tests relative to individual testing, and when $p = 0.01$, this number rises to 80% (Dorfman, 1943). These reductions in tests correspond directly to both financial and

temporal savings, allowing both a cheaper and more rapid identification of infected individuals (Dorfman, 1943). **Figure 1** is inspired by an image from Nebraska Today (Schrage, 2020).

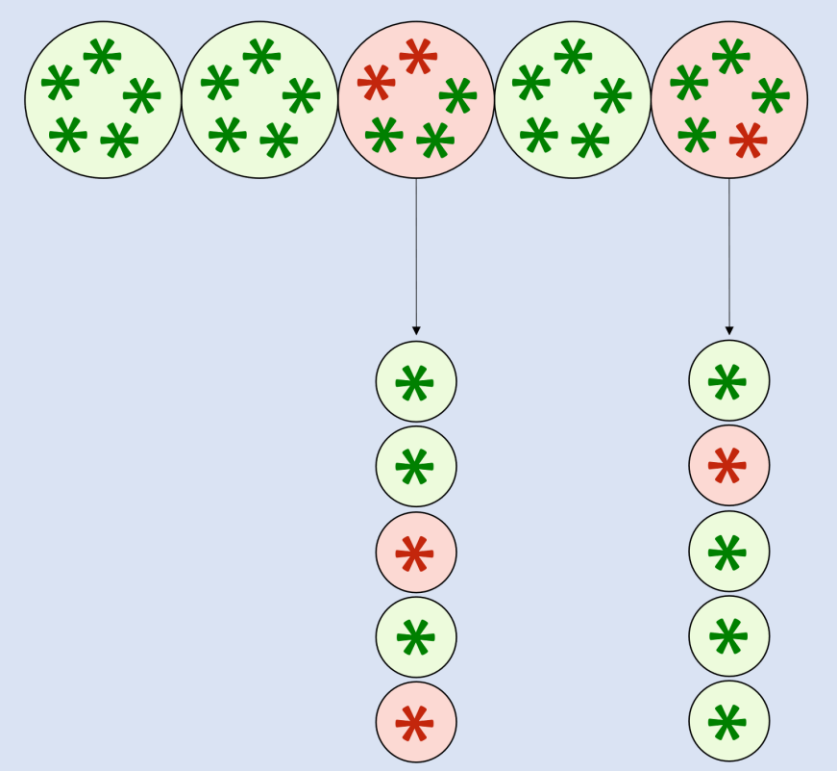

Figure 1. Diagram showing two-level batch testing. Each asterisk represents a person, and each circle represents a test. The dark green asterisks are noninfected, and the dark red asterisks are infected. The circles that are shaded light green represent negative tests, while the circles that are shaded light red represent a positive test.

Multi-Level Batch Testing

Multi-level batch testing, as visualized in **Figure 2**, seeks to improve batch testing further. Within the scope of this research, "multi-level" is specifically used to refer to the method proposed by Keqin Li (Li, 2020). Just like the two-level test, the multi-level test divides a population into groups of size n, and uses the same technique of ruling all members of negative groups as not infected. However, for the remaining groups, the multi-level test then divides them into subgroups of size m, such that $1 \leq m < n$, and repeats the batch testing on the groups of size m. Like before, batches of size m which test negative are not infected; members of batches which test positive are now individually tested. Thus, a "third" layer of batch testing is added in the middle of the original batch testing and the final individual testing, which justifies this method's classification as a multi-level test. This method seems to be generally more efficient than its two-level predecessor; for instance, when $p = 0.01$, this method offers an average of

87% reduction in tests relative to individual testing, which is superior to the 80% reduction noted prior (Li, 2020).

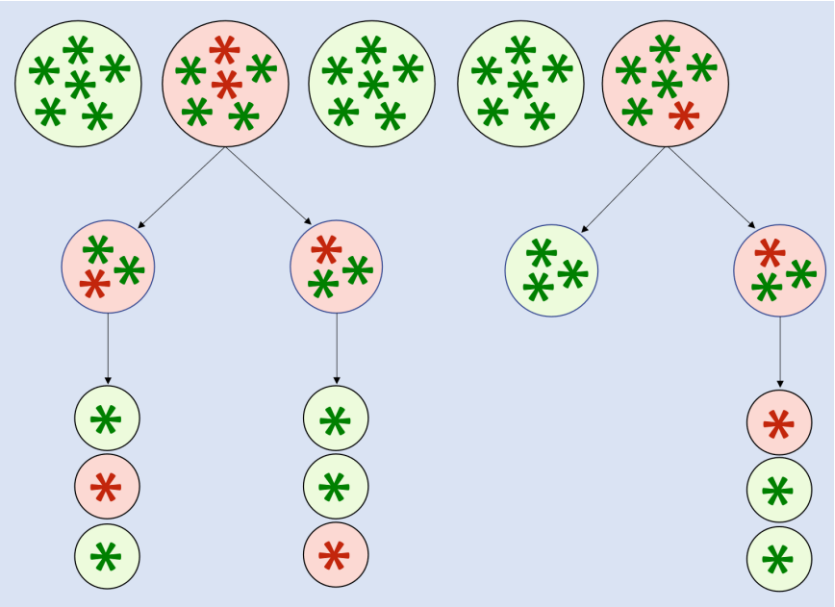

Figure 2. Diagram showing multi-level batch testing. Each asterisk represents a person, and each circle represents a test. The dark green asterisks are noninfected, and the dark red asterisks are infected. The circles that are shaded light green represent negative tests, while the circles that are shaded light red represent a positive test.

Multi Two-Level Batch Testing

The proposed multi two-level test, as visualized in **Figure 3**, also builds upon the methodology of the two-level test, though in a way distinct from the multi-level test. Instead of subdividing groups, this method simply performs a two-level test multiple times. More explicitly, like in the two-level test, each individual is randomly assigned to a group of size n, and these groups are tested. However, unlike the two-level test, this process is repeated; each individual is again randomly assigned to a group of size n, and these new groups are tested. The number of repetitions of this process, r, represents a parameter of optimization. Note when $r = 1$, this method devolves into the two-level method; thus, two-level is a special case of the multi two-level method. After this, each individual will have been randomly assigned to r groups. If just one of an individual's r groups tests negative, the individual is "cleared" and need not be individually tested; however, if all of the r groups test positive, the individual will need to be individually tested. Since only one of r negative batch tests is required to "clear" a member, this

will significantly reduce the quantity of individual testing, thus compensating for the increase in batches tested.

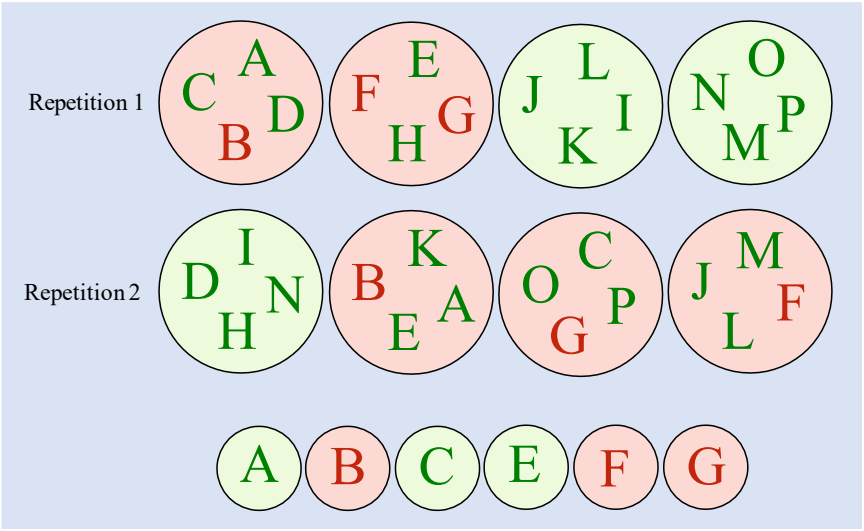

Figure 3. Diagram showing multi two-level batch testing. Each individual letter represents a person, and each circle represents a test. The dark green letters are noninfected, and the dark red letter are infected. The circles that are shaded light green represent negative tests, while the circles that are shaded light red represent a positive test.

Simulation

To quantify and compare the three batch testing methods, a Java simulation was written (McCoy et al., 2021). For a given n and p, it simulates N persons, "infects" (Np) persons with the pathogen, and randomly assigns them to groups of size n. It iterates through various combinations of n and p, and outputs data for statistical analysis. In addition to this, the multi-level test iterates through m such that $1 \leq m < n$, and the multi two-level test iterates through r such that $r \geq 1$. This way, all relevant parameters for each test are addressed. Each batch testing method is run on the N persons, and the number of required simulated tests is recorded. For a given combination of n, p, and m or r where appropriate, this process is run $1{,}000$ times, and the average and standard deviation of the results are output in a SQLite database format. This is imported into R, where it is analyzed, graphics are produced, and comparisons are made.

For the simulation, specific values for the above variables are needed. Based on local COVID-19 testing data for the University of Florida, $N = 5{,}000$ was used to simulate the maximum capacity of the testing lab (Shields, 2021). Additionally, $1 \leq n \leq 100$ (except in multi-level, where $2 \leq n \leq 100$), based on a conservative overestimate of the range for optimal values from two-level (Dorfman, 1943). From preliminary calculations based on the later

derivation, it was determined that $1 \leq r \leq 10$ would be appropriate because the efficiency decreases after $r = 5$ for $p = 0.01$, and after lower values of r for higher values of p.

An algorithm was developed for randomly assigning members to groups of a fixed size. Heuristically, it could be set up that an individual has an $\left(\frac{N}{n}\right)$ chance of being assigned to a specific group, but such a method does not guarantee that all groups have the same fixed size, as some groups will randomly get more members, while others get fewer. Thus, a procedure was implemented to prevent this from occurring. An array of persons was created of size N, and the first $round(Np)$ of them were "infected". This ensures that exactly (or almost exactly, as (Np) is not guaranteed to be an integer) a specified proportion, p, of the individuals is infected. Next, the individuals are shuffled into a random order using the Durstenfeld optimization of the Fisher-Yates shuffling algorithm (Durstenfeld, 1964). Random numbers were generated using the RANLUX++ implementation (Sibidanov, 2017) of the RANLUX random number generator (James & Moneta, 2019). With this randomization, the first n members are assigned to the first group, the second n members are assigned to the second group, and so on. Finally, because $\left(\frac{N}{n}\right)$ is not guaranteed to have a remainder of 0, all remaining members are assigned to their own additional group. This creates randomly assigned groups of a specified size, although the final group may be smaller.

Derivation

Variable Name	Description	Units
$\boldsymbol{N}$	number of people being tested	$person$
$\boldsymbol{n}$	group size	$person/group$
$\boldsymbol{r}$	repetition count	$[unitless]$
$\boldsymbol{p}$	fraction of the population infected	$[unitless]$
$\boldsymbol{q}$	fraction of the population not infected	$[unitless]$
$\boldsymbol{p'}$	probability of forming an infected group of size n	$[unitless]$
$\boldsymbol{q'}$	probability of forming a non-infected group of size n	$[unitless]$
$\boldsymbol{\alpha}$	number of tests per group	$test/group$
$\boldsymbol{\beta}$	number of tests per person	$test/person$
$\boldsymbol{T}$	number of tests	$test$

Table 1. Defining variables used in the derivation.

Assuming a population of sufficiently large size, $N \gg n$, it is expected that a randomly selected individual has probability p of being infected, and a probability q of not being infected.

$$q = 1 - p$$

Thus, randomly selecting a group of size n, in which none of the members are infected, has the probability q'.

$$q' = q^n$$

Correspondingly, the probability of selecting a group of size n with at least one infected member is p', which is the complement of q'.

$$p' = 1 - q' = 1 - (1 - p)^n$$

Finally, the expected number of tests, $E(T)$, when using a single repetition, is equal to the sum of the number of groups which are tested, $\left(\frac{N}{n}\right)$, and the number of individuals in groups which are expected to be infected.

$$E(T) = \alpha\frac{N}{n} + \beta\left(\frac{N}{n}p'\right)n = N\left(\frac{\alpha n + \beta}{n} - \beta(1 - p)^n\right)$$

The constants α and β are introduced to ensure that the formula outputs correct units. For this study, $\alpha = 1\ test/group$ and $\beta = 1\ test/person$. When this substitution is made,

$$E(T) = N\left(\frac{n + 1}{n} - (1 - p)^n\right)$$

which perfectly aligns with Dorfman's two-level method (Dorfman, 1943). However, future studies may wish to use different values of α and β to account for false positives and negatives, or other aspects which go beyond the scope of this paper.

Given this derivation for Dorfman's two-level method, it is now appropriate to extend it to instances of multiple repetitions; indeed, Dorfman's method is a special case of the multi two-level method when only a single repetition is performed. This number of repetitions, r, is a fundamental parameter of the multi two-level method.

For each test, $r\left(\frac{N}{n}\right)$ groups are generated, as each repetition creates $\left(\frac{N}{n}\right)$ groups. Thus, $\alpha r\left(\frac{N}{n}\right)$ tests must be performed for each group. Additionally, in each repetition, all members in non-infected groups are "cleared," which means they are not infected and do not need to be individually tested. However, an individual can be cleared multiple times, and some individuals will remain "uncleared" if they are in an infected group for each repetition. Consider a single round. Let A be the event that an individual is in a non-infected group of size n, and I be the event that the individual is not infected. Then,

$$P(A) = P(A, I) + P(A, I')$$

However, it is impossible for an infected individual to be in a non-infected group, so $P(A, I) = 0$. Thus,

$$P(A) = P(A, I') = P(A|I') * P(I')$$

For this individual, $P(I') = q$. Additionally, the probability that an individual is in a non-infected group, given that the individual is not infected, is equivalent to the probability that all other $n - 1$ members are not infected, which is q^{n-1}. Thus,

$$P(A) = q^n = q'$$

Thus, as an individual has a q' chance of being in a non-infected group, that individual has a p' chance of being in an infected group. It is apparent that Nq' individuals will be "cleared" in each repetition, which leaves Np' individuals "uncleared."

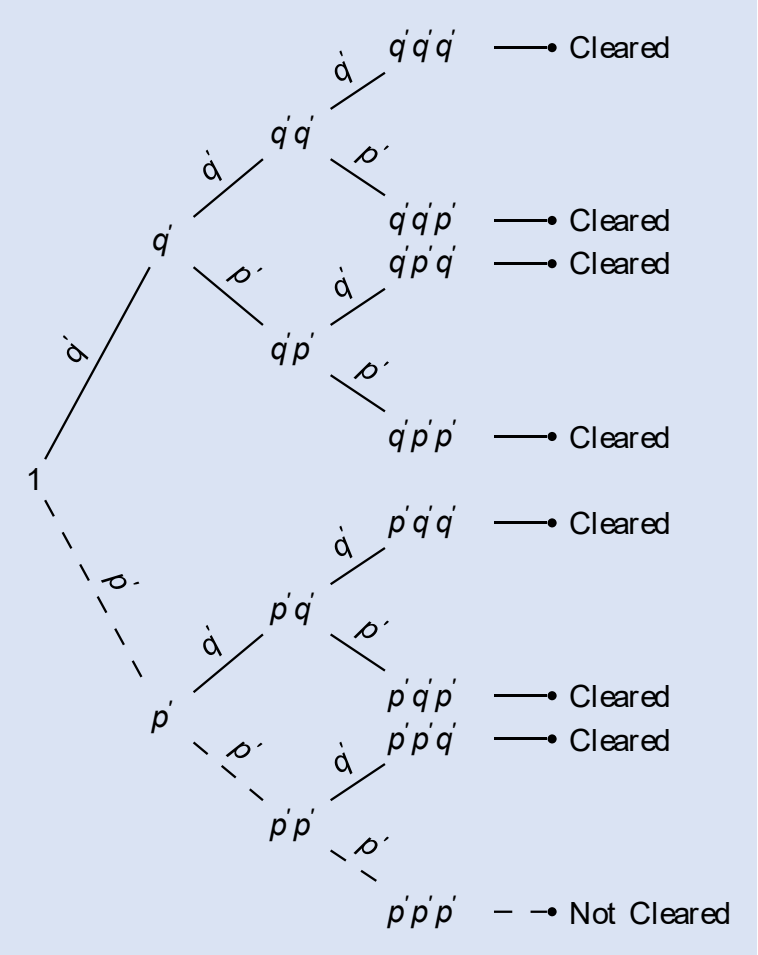

Figure 4. Probability tree for $r = 3$. Note that the only instance where an individual is not "cleared" is when they are placed in r successive infected groups, which has a probability of $(p')^r$. This instance is denoted with a dashed line, and its probability is $(p')^3$ for the example in this figure.

It is evident from **Figure 4** that the probability of being in an infected group for every repetition is $(p')^r$. Thus, the number of people who would have to be individually tested is $N(p')^r$. Therefore, the number of additional tests that must be performed for each individual who was never "cleared" is $\beta N(p')^r$.

Finally, it is possible to calculate the expected total number of tests for the multi two-level method by adding together the number of tests needed for each group and for each individual who was never "cleared."

$$E(T) = \alpha r\left(\frac{N}{n}\right) + \beta N(p')^r$$

As an additional note, this equation simplifies to Dorfman's equation when $r = 1$, which was previously stated.

Results and Discussion

Fraction of tests saved refers to $f = 1 - \left(\frac{T}{N}\right)$, which corresponds to the proportion of tests saved for the selected method when compared to the number of tests required for individual testing. **Figures 5, 6, and 7** plot fraction of tests saved for their respective methods at $p = 0.01$.

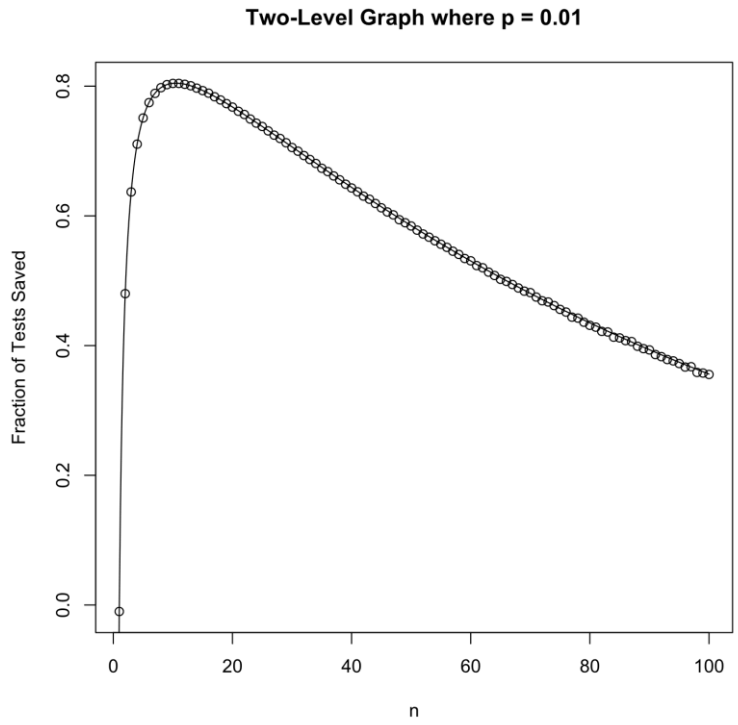

Figure 5. Two-level method graph, where $p = 0.01$.

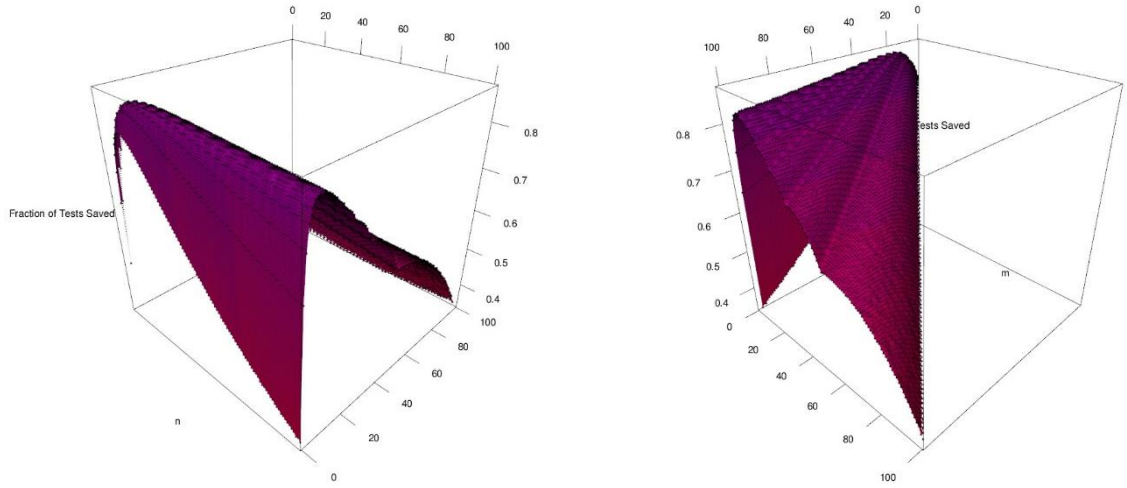

Figure 6. Multi-level method 3D plot of simulation data, where $p = 0.01$. Click here for interactive graph.

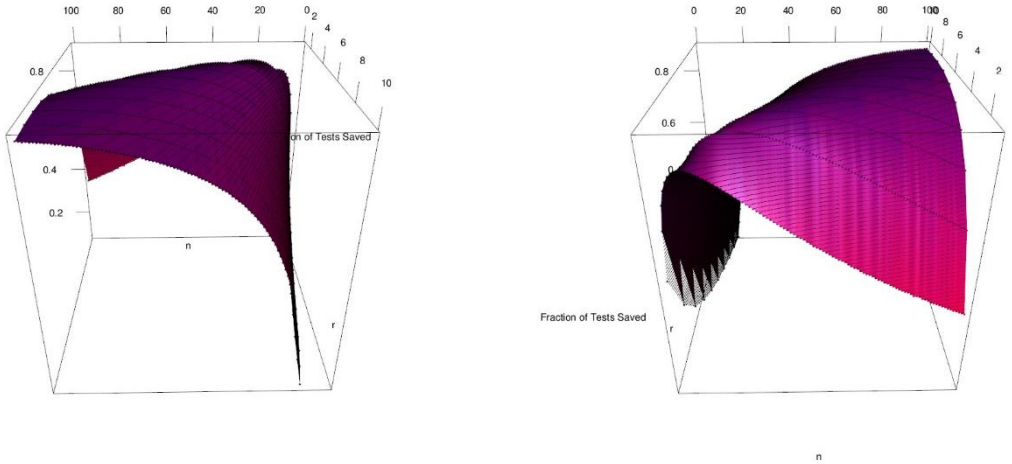

Figure 7. Multi two-level method 3D plot of simulation data, where $p = 0.01$. Click here for interactive graph.

Figures 5, 6, and 7 show the distribution of efficiencies for the three testing methods over the simulated range of their respective parameters. Any data point with a fraction of tests saved value less than 0 was omitted, as a negative value indicates inefficiency. The maximum values for fraction of tests saved in **Figures 5, 6, and 7** occur at $(n = 11, f = 0.8042)$, $(n = 25, m = 5, f = 0.8665)$, and $(n = 65, r = 5, f = 0.8889)$, respectively; these values are also reflected in **Table 2**. Although these figures may look different, they show the output of the simulation for the batch testing methods and demonstrate the maximum efficiency of each method at $p = 0.01$.

Additionally, **Figure 5** demonstrates how closely the simulated data for the two-level method lines up with the expected theoretical values. The solid line represents the expected theoretical values based on Dorfman's equation $E(T) = N\left(\frac{n+1}{n} - (1-p)^n\right)$, and the hollow circles are the simulation data. Theoretical values were omitted from **Figures 6 and 7** for visual clarity.

	Two-Level			Multi-Level				Multi Two-Level			
p	n	Fraction of Tests Saved	Standard Deviation	n	m	Fraction of Tests Saved	Standard Deviation	n	r	Fraction of Tests Saved	Standard Deviation
0.01	11	0.8042	3.1005E-03	25	5	0.8665	2.4960E-03	65	5	0.8889	3.8934E-03
0.05	5	0.5737	4.1866E-03	9	3	0.6230	4.2624E-03	13	3	0.6265	6.1073E-03
0.10	4	0.4060	5.2743E-03	9	3	0.4132	6.8362E-03	7	2	0.4167	7.5709E-03
0.15	3	0.2809	4.8741E-03	96	3	0.2705	4.6591E-03	3	1	0.2807	4.8118E-03
0.20	3	0.1785	5.9267E-03	99	3	0.1684	5.8898E-03	3	1	0.1787	5.6312E-03
0.25	3	0.0885	6.3498E-03	99	3	0.0780	6.2103E-03	3	1	0.0881	6.3824E-03
0.30	3	0.0094	6.4143E-03	99	3	-0.0010	6.7164E-03	3	1	0.0094	6.8017E-03

Table 2. Table showing the numeric values from the simulation of two-level, multi-level, and multi two-level methods, which correspond to the red, blue, and green shading, respectively. Values beyond $p = 0.30$ were omitted as the fraction of tests saved becomes negative, rendering the methods useless, as seen in **Figure 8**.

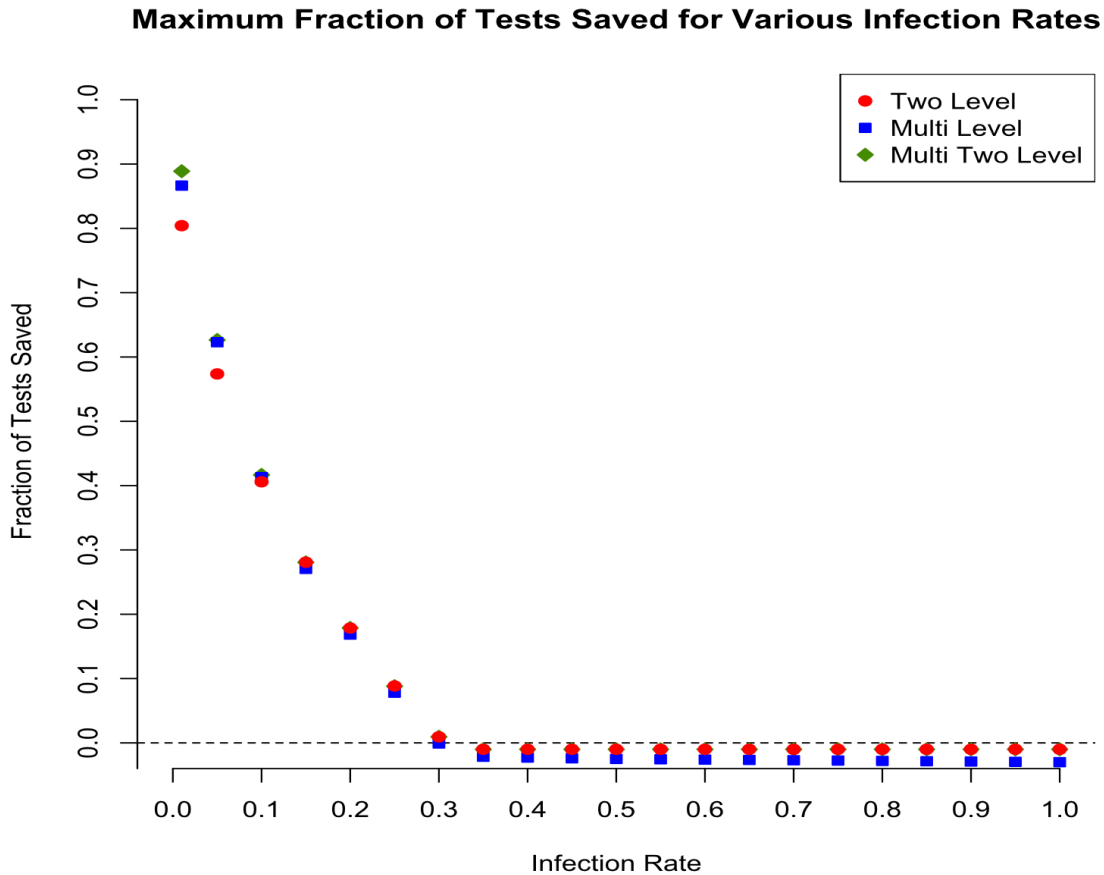

Figure 8. Shows comparison between all testing methods at multiple infection rates. The colors in the legend correspond to the shading in **Table 2**.

p	Tukey Critical Value	Multi Two -Level vs. Two-Level	Multi Two -Level vs. Multi-Level	Multi-Level vs. Two-Level
0.01	0.000337105	0.0846398	0.0223892	0.0622506
0.05	0.000517274	0.052805	0.0034634	0.0493416
0.10	0.000695261	0.0106922	0.0034294	0.0072628
0.15	0.000501522	0.0001976	0.0101304	0.010328
0.20	0.000610046	0.0001598	0.0102828	0.010123
0.25	0.000662187	0.0003978	0.0100826	0.0104804
0.30	0.000696959	0.0000036	0.0103578	0.0103614

Table 3. Tukey critical values for comparison of the optimal combinations at each p where the methods are efficient at $\alpha = 0.01$. Cells shaded green shows statistical significance, while cells shaded red have no statistical significance. Values where $p > 0.30$ were omitted due to inefficiency beyond this level.

The multi two-level method's outperformance of current methods at 1% infection rate indicates that it is a viable improvement. As **Table 3** demonstrates, multi two-level outperforms two-level by a statistically significant margin up until $p > 0.10$, at which point multi two-level devolves into the two-level case as $r = 1$, as seen in **Table 2**. Multi two-level outperforms multi-level by a statistically significant margin up until $p = 0.15$. However, when $p \geq 0.20$, as seen in **Table 2**, the assumption that $1 \leq n \leq 100$ fails when applied to multi-level as $n = 99$ is suspiciously close to the upper boundary. This implies that the optimal value of n could be greater. For example, through analysis of a plot of multi-level at $p = 0.20$, the efficiency of multi-level eventually exceeds that of multi two-level after $n \approx 6{,}000$, which could either be feasible or not depending on the population size because of the constraint $N \gg n$. Thus, when $p \geq 0.20$, it is possible for multi two-level to be more realistically performant depending on N. When $p > 0.30$, all methods become inefficient because the fraction of tests saved drops below 0, so there is no value in comparing them, which is visually depicted in **Figure 8**.

An inherent problem exists when the population is not evenly divisible by the group size. Some papers omit this issue by allowing fractional individuals, but in the simulation, individuals are treated as integer values. This issue was handled by assigning all remaining members to a new group of a size smaller than the other groups, but other valid procedures could exist and would most likely impact the real-world efficiency of the methods.

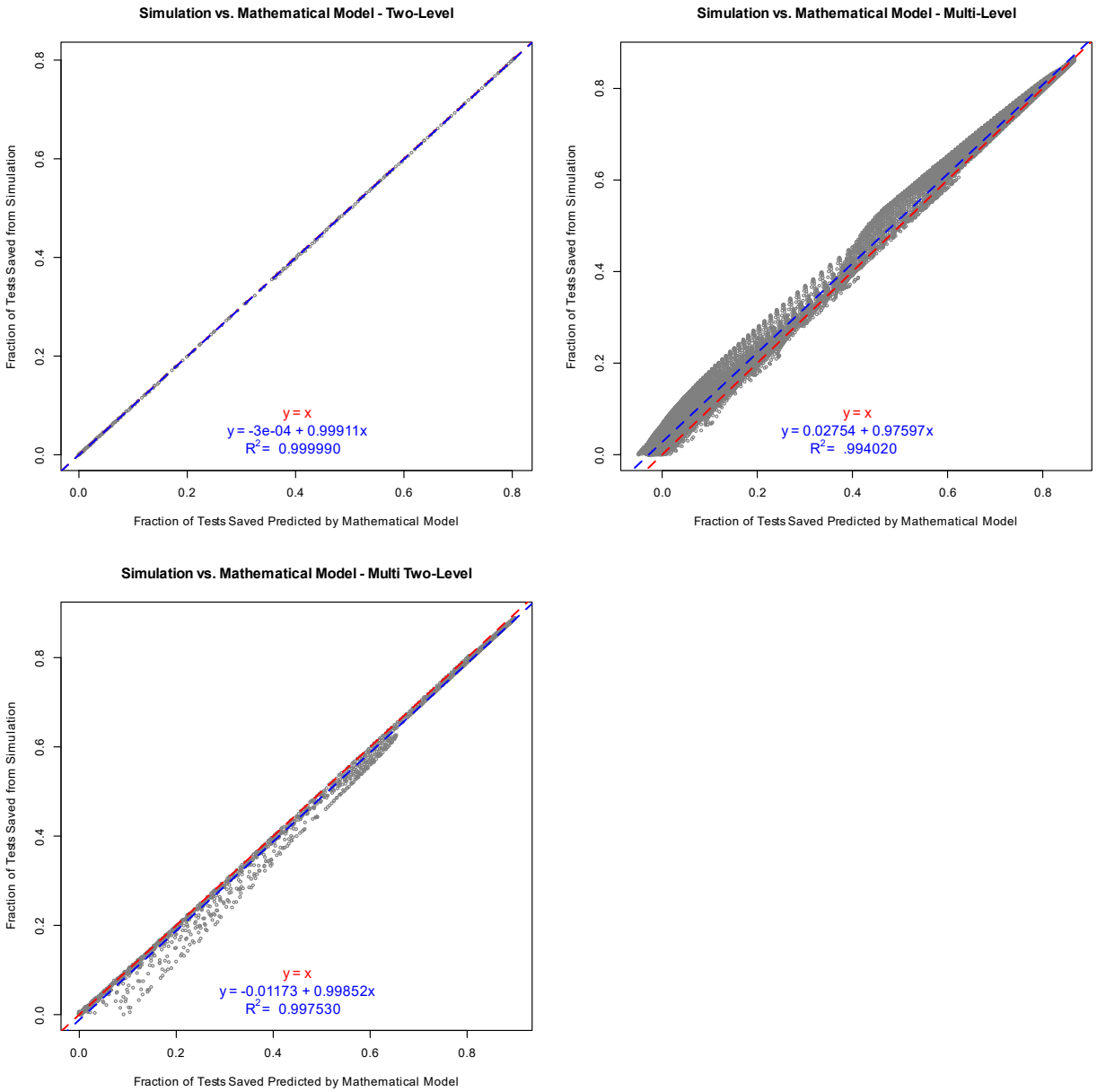

Figure 9. Plot of simulation output versus mathematical model for two-level, multi-level, and multi two-level. The regression line is plotted in blue, and the identity line is plotted in red.

For **Figure 9**, all values where the simulation is inefficient, or where the fraction of tests saved becomes negative, were excluded. The identity line corresponds to an exact match between the mathematical model and simulation, and any point below the red line represents inefficiency in the simulation relative to the mathematical model.

The mathematical model used in **Figure 9** to calculate fraction of tests saved for two-level is $1-\left(\frac{n+1}{n}-(1-p)^n\right)$ (Dorfman, 1943), for multi-level is $1-\left(\frac{q^n+\left(n\left(\frac{1}{m}+\frac{1-q^m}{1-q^n}\right)+1\right)(1-q^n)}{n}\right)$ (Li, 2020), and for multi two-level is $1-\left(\frac{r}{n}+(1-q^n)^r\right)$. For each method, there is a high correspondence between the simulation results and the mathematical models. As $R^2=0.999990$ in **Figure 9** for the two-level method, there is virtually no difference between the mathematical model and simulation. For the multi-level method, much more noticeable variation exists, with the simulation both outperforming and underperforming the model at certain cases; however, there is a clear equivalence between them as indicated by $R^2=0.994020$. For the multi two-level method, there is also a clear equivalence indicated by $R^2=0.997530$; however, the model only ever outperforms the simulation. Finally, a probable reason for the difference may be the rounding associated with population sizes that are not evenly divisible by group sizes, but this does not explain the outperformance of the simulation for multi-level, which requires further investigation.

Limitations

Current technology is advanced enough to where false positive and negative COVID-19 results are rare, especially if tested when symptoms are present (Shmerling, 2021). Due to their low occurrence, false positives and negatives were assumed to be negligible in this paper, but future studies may take these factors into consideration as they may have a nontrivial effect on the results. Moreover, with current infrastructure in pathology laboratories, it may be more manually intensive to perform the repetitions required for the multi two-level method, although a cost analysis would have to be performed to corroborate that statement. A potential increase in logistical complexity may render it economically unviable.

Conclusion

The multi two-level methodology for batch testing is a statistically significant improvement over current testing procedures with 89% efficiency relative to individual testing at a 1% infection rate. It should be utilized whenever a particular illness must be identified in a population with an infection rate around one percent, assuming sufficient infrastructure. The data supports the hypothesis that multi two-level is most efficient for a certain value of p. The novel

method is specifically applicable to the COVID-19 pandemic, as this will allow the medical community to better meet the demand from the public for COVID-19 tests and decrease community spread of COVID-19, as asymptomatic individuals can quarantine more quickly once they receive their positive test result (Chen et al., 2020). Moreover, this research may be extended to any disease that can be tested in groups because the same principles of batch testing apply to any easily transmittable disease. With ever increasing risk for future pandemics, such as the proliferation of global travel, and the melting of ice sheets which reintroduces DNA viruses, research into the most optimal testing method is crucial to mitigating the deleterious effects of future pandemics (Legendre et al., 2014).

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