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Smart Pooling: AI-powered COVID-19 Informative Group Testing

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ABSTRACT

Massive molecular testing for COVID-19 has been pointed out as fundamental to moderate the spread of the pandemic. Pooling methods can enhance testing efficiency, but they are viable only at low incidences of the disease. We propose Smart Pooling, a machine learning method that uses clinical and sociodemographic data from patients to increase the efficiency of informed Dorfman testing for COVID-19 by arranging samples into all-negative pools. To do this, we ran an automated method to train numerous machine learning models on a retrospective dataset from more than 8,000 patients tested for SARS-CoV-2 from April to July 2020 in Bogotá, Colombia. We estimated the efficiency gains of using the predictor to support Dorfman testing by simulating the outcome of tests. We also computed the attainable efficiency gains of non-adaptive pooling schemes mathematically. Moreover, we measured the false-negative error rates in detecting the ORF1ab and N genes of the virus in RT-qPCR dilutions. Finally, we presented the efficiency gains of using our proposed pooling scheme on proof-of-concept pooled tests. We believe Smart Pooling will be efficient for optimizing massive testing of SARS-CoV-2.

Introduction

COVID-19 is an acute respiratory illness caused by the novel coronavirus SARS-CoV-2^{1,2}. It has rapidly spread to most countries, causing 182 million confirmed infections and over 3.9 million deaths as of June 29th 2021³. In order to contain the ongoing pandemic, countries have rushed to implement massive testing to control the spread of the disease by identifying and isolating carriers. Massive testing is a fundamental strategy to curb the disease⁴, mainly due to its asymptomatic transmission⁵. Large-scale testing is costly and requires scarce reagents. As there is a global need to make testing more accessible to larger populations, strategies to increase the number of people that can be tested with the same amount of test kits are urgent.

Dorfman proposed pooling methods⁶ to diagnose syphilis among the US military during World War II by combining samples from multiple soldiers in a single test tube. Different pooling strategies, such as Dorfman's two-stage pooling^{6,7} and matrix pooling⁸, offer higher efficiency gains for different combinations of test sensitivity and disease prevalence.

Existing works have demonstrated that using prior information to exclude samples likely to be positive from pools can increase testing efficiency. In informative group testing, as stated by Bilder *et al.*, the different probabilities of individuals testing positively are exploited by grouping their samples into pools that are more likely to be all negative⁹. McMahan *et al.* proposed to threshold individuals as "high" or "low risk" and to use risk-specific algorithms while simultaneously identifying pool sizes to minimize the expected number of tests¹⁰. Taylor *et al.*¹¹ used a complementary microscopic examination of samples in the grouped detection of malaria and Lewis *et al.*¹² used clinical information in patients' forms in the grouped

detection of chlamydia and gonorrhea.

Previous studies in group testing aim to reduce the number of tests by finding the optimal design choices such as the pool sizes and the number of stages, and the assignment of specimens to the pools, as explored in¹³ by estimating the probability of disease positivity. Arahamian *et al.* proposed to maximize the classification accuracy by reducing the number of false negatives under a budget constraint and evaluated an adaptive risk-based pooling scheme on Chlamydia screening in the US¹⁴. Additionally, pooling has also been used in genetic sequencing^{15,16} and drug screening¹⁷, among other applications.

In this paper, we present Smart Pooling, a machine learning (ML) method for optimizing massive testing of SARS-CoV-2. We exploit clinical and sociodemographic information of samples to estimate their posterior probability of testing positive for COVID-19. As Fig. 1b shows, our method uses these probabilities to arrange samples into pools that maximize the probability of yielding a negative result within the majority of pools. Samples predicted to be positive are tested individually, reducing the number of kits required to diagnose the same amount of samples. Our method can be used jointly with existing pooling algorithms to enhance their efficiency. Smart Pooling can be thought of as an informative group testing¹⁰ in which the patients' probability of positivity is learned from clinical data through state-of-the-art Artificial Intelligence (AI) techniques.

Empirical tests have confirmed the ability of pooled sampling to reliably detect SARS-CoV-2 in a pool comprising one positive and up to 31 negative specimens^{18,19}. Moreover, preliminary trials show that pools of 5²⁰ and 10²¹ samples can increase efficiency without strongly compromising sensitivity. At the same time, Bayesian non-adaptive pooling schemes demonstrate increased efficiency for low prevalences in experiments in vitro²². Pooling strategies could facilitate detection of early community transmission²³. However, they begin to fail as disease prevalence increases²⁴, as shown in Fig. 1a.

Figure 1

In the context of COVID-19, AI-based models have been used to classify the novel virus from its genetic sequencing²⁵, to support diagnosis from CT scans²⁶, to assist clinical prognosis of patients²⁷, and to forecast the evolution of the pandemic²⁸. Menni *et al.*²⁹ used regression models trained with patient-reported symptoms and laboratory test results to predict infection. Using these strategies for diagnostics risks compromising sensitivity and confidence. Smart Pooling does not seek to replace current molecular testing but assists it by improving its efficiency.

Smart Pooling is easily adaptable to any pooling procedure. We propose a five-step pipeline between the laboratory

procedures and the Smart Pooling analysis to use Smart Pooling for the desired pooling strategy. Fig. 1c illustrates this process. First, the laboratory acquires samples and complementary data from patients. Secondly, the Smart Pooling platform processes the data and triages the samples. Thirdly, samples are pooled according to this ordering in the laboratory. Then, molecular tests are run on the ordered pools until there is a diagnosis for each sample. Lastly, the laboratory feeds the results of the tested samples into the Smart Pooling platform to improve the model continuously.

Smart Pooling decreases the expected number of tests per specimen to 0.36 and 0.94 at a disease prevalence of 5% and up to 50%, respectively, including a regime in which Dorfman's testing procedure no longer offers efficiency gains compared to individual testing (see Fig. 2). In this context, we define efficiency as the inverse of the expected number of tests per specimen. Additionally, we calculate the possible efficiency gains of one- and two-dimensional two-stage pooling strategies and present the optimal strategy for disease prevalences up to 25%. We discuss practical limitations to conduct pooling in the laboratory. Pooled testing has been a theoretically alluring option to increase diagnostics coverage since its proposition by Dorfman. Although there are examples of successfully using pooled testing to reduce diagnostics costs, its applicability has remained limited because efficiency drops rapidly as prevalence increases. Not only does our method provide a cost-effective solution to increase the coverage of testing amid the COVID-19 pandemic, but it also demonstrates that artificial intelligence can be complementary to well-established techniques in the medical praxis. To ensure reproducibility and promote further research, we make a portion of our dataset available for public use in the supplementary information.

Figure 2

Results

Efficiency gains from Smart Pooling

We identify that using complementary information to arrange pools can improve the efficiency of testing. Fig. 2 show Smart Pooling's simulated expected number of test per specimen at disease prevalence ranging from 5% up to 50% in the *Patient Dataset* and from 5% up to 25% in the *Test Center Dataset*, with a fixed pool size of 10. We compare our AI-enhanced Dorfman's testing against its original counterpart and the individual testing procedure. Note that these ranges are higher than those applicable for optimal standard pooling methods. Overall, Smart Pooling's efficiency outperforms that obtained by simulating Dorfman's two-stage pooling and individual testing.

We rank samples according to the model's predicted probability of yielding a positive result. Fig. 3 shows how the simulated predictions from the AI method have most of the positive samples at the top of the ranking. The key idea of Smart Pooling is that it maintains the prevalence artificially low, even under scenarios with a high overall prevalence of COVID-19, by triaging samples from a priori complementary information before the pooling takes place.

Figure 3

Smart Pooling is not limited to Dorfman's testing procedure; it can be coupled with multiple pooling strategies and improve efficiency regardless of the strategy. Supplementary Fig. 1 shows Smart Pooling's simulated expected number of tests per specimen with a fixed pool size of 10, an adaptive pooling strategy based on the optimal strategies explored in Section , Dorfman's standard testing, and the individual testing. When coupled with Smart Pooling, these alternatives reduce the expected number of tests per specimen, thus are more efficient than Dorfman's two-stage pooling and individual testing.

Optimal pooling strategies

In this section, we find the pooling protocols that maximize efficiency for a given prevalence p , assuming a fixed bound c on pool size. The efficiency, as mention previously, is defined as the inverse of the expected number of tests per specimen.

We explored the following two kinds of pooling protocols:

1. Dorfman's pooling protocols⁶: Given m samples, we make a single pool with all of them. We denote this protocol by S_m .
2. Matrix pooling protocols⁸: Given a collection of $m \times n$ samples, we place them into a rectangular $m \times n$ array. We create pools by combining samples along the rows and columns of this array (for a total of $m + n$ pools). In the second phase, we test each sample at the positive columns and rows intersection individually. We denote this protocol by $S_{m \times n}$.

Quantifying pooling efficiency

We define pooling efficiency as our main tool to quantitatively compare different pooling protocols, which depends on the prevalence p of the sample. Assuming independence among patients, pooling efficiency can be computed analytically^{6,8}:

1. For Dorfman's pooling S_m the efficiency is given by

$$H(p) = \frac{1}{\frac{1}{m} + 1 - (1-p)^m}$$

2. For the matrix pooling protocol $S_{m \times n}$ the efficiency is given by

$$H(p) = \frac{1}{\frac{1}{m} + \frac{1}{n} + p + (1-p)(1 - (1-p)^{m-1})(1 - (1-p)^{n-1})}$$

The efficiency functions above show the key property behind pooling: at low prevalences, efficiency can be considerably greater than one, but as the prevalence increases, $H(p)$ decreases, with efficiency gains becoming negligible after prevalences around 30%. Fig. 4 illustrates these efficiency gains (and how they vanish) for two-stage pooling.

Optimizing pooling strategies

Practice in the laboratory constrains the maximum pool size. In the context of COVID-19, we will focus on the cases $c = 5$ and $c = 10$ since these are the most useful in practice (see supplementary information for details). Finding the optimal pooling strategies for a given prevalence equals finding the pooling protocol of maximum efficiency by comparing the values of $H(p)$ for the different protocols³⁰. The maximum efficiency is obtained by minimizing the expected number of tests per specimen. Fig. 5 shows the expected number of tests per specimen curves of the best pooling protocols of the form S_m and $S_{m \times n}$ with maximum pool size $c \leq 10$ and $c \leq 5$. There is an optimal pooling strategy for each prevalence; Table 1 shows the optimal protocols and their respective intervals of optimality when $c = 10$ and $c = 5$ respectively. Even while using optimal protocols, it is clear that efficiency gains quickly disappear as the prevalence increases (effectively vanishing when $p \geq 30.7\%$), which we improve by using Smart Pooling.

Sensitivity experiments in diluted samples

To test for possible sensitivity reduction from sample dilution in a realistic setting, we performed the following experiment: We diluted a positive sample successively $1.4 \times$ ten times in negative samples, from an expected C_t of 38 to 43, in 8 replicates. The diluted samples were processed using the U-TOP COVID-19 Detection Kit (SeaSun Biomaterials)³¹ with the recommended parameters, except that we increased the number of cycles to 43. The average C_t (for the wells that give a reading) increases with dilution but, deviates from the expected value (the observed one is lower), and the number of wells with no reading increases from none, in the initial dilutions, to most, in the higher dilutions. We show the expected average C_t and fraction of wells with no reading for markers N and ORF1ab in Table 2.

We combined the data for both genes and linearly interpolated it to convert this value into an estimate of the number of false negatives. We tabulated the measured C_t s of the positive samples from 20,000 tests (2,678 samples) and the expected C_t s for different dilutions. Assuming the threshold C_t for testing individual samples in a pool is adjusted according to the dilution,

Figure 4

the number of samples that would give no reading if in a pool with only negatives for a given dilution can be estimated by convolving the distribution of C_{t_s} with the failure rate for the resulting C_{t_s} after dilution.

The estimate is an upper bound since the calculations are performed for each marker gene separately. The criterion used in practice is to consider a sample positive if C_t is below 38. The latter does not consider the probability that the sample could be in a pool with other positives. For our experiment, we limited the number of cycles to 43. However, a higher number of cycles could give a better performance for more diluted samples. Furthermore, pooling schemes that include the same sample in multiple pools, such as the 8x2 scheme, are less susceptible to this type of error. Table 3 shows the false-negative rate, obtained as explained for single pools of up to 16 samples for markers N and ORF1ab.

Proof-of-concept Smart Pooling Implementation

This section describes the deployment of Smart Pooling in the GenCore laboratory of the Universidad de Los Andes in Bogotá, Colombia. The project COVIDA, an active surveillance program, provides samples that we use to implement Smart Pooling. COVIDA's two main objectives are: first, to trace new cases of infection with an active surveillance strategy. Second, the project supports decision-makers by providing relevant information for developing public health policies in Bogotá. Our proof-of-concept experiment in the GenCore laboratory lasted seven days. On average, GenCore tests 300 samples from COVIDA every day. More than 4,700 tests were processed during our experiment using the Smart Pooling methodology, and 2,012 kits were saved using Smart Pooling instead of individual testing.

For this proof-of-concept stage, the GenCore laboratory implemented Smart Pooling with a pool size of 2. Taking into account that in this stage, we use scarce laboratory resources (equipment and personnel), it was not feasible to evaluate the

Figure 5

performance of Dorfman's testing in the exact same samples for a real-life comparison with Smart Pooling. However, we simulated the theoretical performance of Dorfman's testing for each day's samples in the proof-of-concept.

Fig. 2 shows the comparison between the performance of Smart Pooling's results after implementation and Dorfman's testing simulation. Each point on the graph represents the expected number of tests per specimen for a day in the proof-of-concept stage. The x-axis depicts the incidence of the corresponding day. We show that the performance of Dorfman's testing is comparable with Smart Pooling; these results are expected due to the low prevalence of the samples in the COVIDA project. The prevalence in the samples is lower than 10% as a result of the project's active surveillance nature. Nevertheless, implementing Smart Pooling provides further benefits such as an epidemiological overview of the daily state of the pandemic and clear identification of high-risk patients to prioritize the processing of their samples. We also demonstrate that the performance of Smart Pooling follows our initial observation, which pointed out that, regardless of the prevalence, Smart Pooling has higher efficiency than individual testing. Even though the proof-of-concept was done under a limited pool size of 2, our results prove that Smart Pooling can be successfully implemented in laboratories, and it can be easily scaled up for other testing facilities with capacities of performing pooling with larger pool sizes.

Discussion

Our computational experiments show that, regardless of the level of granularity of the data available (Fig. 2), Smart Pooling obtains efficiency gains for all simulated prevalences up to 25% for the *Test Center Dataset* and for the *Patient Dataset* up to 50% when comparing against the standard Dorfman's testing. For instance, with Smart Pooling at a prevalence of 10% and an expected number of tests per specimen around 0.5, even with coarse data in the test center approach, the estimated number of patients that could be tested with the same number of tests is doubled compared to individual testing. These results show that Smart Pooling could be viable in various settings and does not depend on the availability of rich complementary data.

Fig. 3 illustrates the working principle of Smart Pooling: it enhances efficiency by artificially reducing the incidence in the samples arranged in pools by testing individually the samples most likely to yield a positive result.

The machine learning model takes advantage of complementary data to compute the probability that the sample results positively. For the *Patient Dataset*, this could come from reported symptoms or the knowledge that the patient belongs to a particular group (for instance, being a health worker). For the *Test Center Dataset*, the machine learning model could be exploiting underlying correlations in the samples³². In other words, Smart Pooling could be seen as the assembly of pools with correlated samples where the Dorfman's protocol's independence assumption in the underlying binomial distribution no longer holds (within the pool of *correlated* samples)³². Samples in this dataset were acquired during strict measures limiting mobility in the city of Bogotá. People tested at the same center likely shared epidemiological factors. The model could also be learning the different probability distributions of samples being positive in various city locations.

Smart Pooling enhances but does not replace molecular testing. Smart Pooling uses AI to enhance the performance of well-established diagnostics. It demonstrates that data-driven models can complement high-confidence molecular methods. Its robustness to the variability of the available data, prevalence and model performance, and its independence of pooling strategy, make our work compelling to apply at large scales. Additionally, Smart Pooling's continuous learning should make it robust to

Table 1

$c = 10$			$c = 5$		
Pooling protocol	Prevalence Interval (%)		Pooling protocol	Prevalence Interval (%)	
	Lower bound	Upper Bound		Lower bound	Upper Bound
S_{10}	0.00	1.25	S_5	0.00	6.625
S_9	1.25	1.375	S_4	6.625	12.375
$S_{10 \times 10}$	1.375	4.875	S_3	12.375	25.00
$S_{9 \times 10}$	4.875	5.25			
$S_{9 \times 9}$	5.25	5.875			
$S_{8 \times 9}$	5.875	6.375			
$S_{8 \times 8}$	6.375	7.375			
$S_{7 \times 8}$	7.375	8.125			
$S_{7 \times 7}$	8.125	9.625			
$S_{6 \times 7}$	9.625	10.875			
$S_{6 \times 6}$	10.875	12.125			
S_4	12.125	12.375			
S_3	12.375	25.00			

Prevalence intervals and their respective optimal pooling strategy. S_m are single pooling protocols and $S_{m \times n}$ are matrix pooling protocols.

our understanding of the pandemic and its evolution.

Smart Pooling could ease access to massive testing. This pandemic has presented challenges to all nations. As the number of infected people and contagion risk increases, more testing is required. However, the supply of test kits and reagents cannot cope with the demand, with most countries not able to perform 0.3 new tests per thousand people³. Adopting Smart Pooling could translate into more accessible massive testing. In the case of Colombia, this could mean testing 50,000 samples daily, instead of 25,000, in mid-July 2020³³. If deployed globally, Smart Pooling truly has the potential of empowering humanity to respond to the COVID-19 pandemic and save thousands of lives. It is an example of how AI can be employed to bring social good.

Methods

We used an automated Machine Learning (ML) method³⁴ to train several models on a retrospective dataset of RT-qPCR tests for SARS-CoV-2, including clinical and sociodemographic information of samples. We used the predictions of the best ML model to simulate the Smart Pooling process. We also found the theoretical optimal pooling protocols for a given prevalence. Then, we evaluated the sensitivity of pooled RT-qPCR SARS-CoV-2 testing for dilutions between 0x and 28.9x. Finally, we executed proof-of-concept experiments of our proposed AI-assisted Dorfman's pooled testing. This study was approved by the ethics committees at Universidad de los Andes and the Health Authority of Bogotá after the due revision process, and it was performed following the Declaration of Helsinki regulations. As an anonymized retrospective study, the need for informed consent was waived.

Dataset

The data we used correspond to the molecular tests conducted by Universidad de Los Andes for the Health Authority of Bogotá, Colombia. We tested samples individually following the Berlin Protocol³⁵ and the protocol for the U-TOP™ COVID-19 Detection kit from Seasun Biomaterials³¹, before and after April 18th respectively. Our dataset consists of two different data groupings, representing the diversity of available data, the Patient Dataset and the Test Center Dataset. The goal of the Patient Dataset is to capture the epidemiological and social characteristics of individuals that may lead to infection of COVID-19. On the other hand, the goal of the Test Center Dataset is to depict the temporal evolution of the pandemic at a group level. Our work has the ethics committee's approval at Universidad de Los Andes, and, as an anonymized retrospective study, the need for informed consent was waived.

Table 2

Dilution	ORF1ab				N		
	Expected C_t	Average C_t	Fail fraction		Expected C_t	Average C_t	Fail fraction
0X	38.4	39.0	0.00		38.0	37.5	0.00
1.4X	38.9	39.0	0.13		38.5	38.0	0.00
2X	39.4	40.1	0.00		39.0	38.5	0.00
2.7X	39.9	40.5	0.00		39.5	38.9	0.00
3.8X	40.3	40.9	0.63		39.9	39.3	0.13
5.4X	40.8	42.1	0.67		40.4	39.9	0.33
7.5X	41.3	41.6	0.50		40.9	399.0	0.50
10.5X	41.8	-	1.00		41.4	40.2	0.57
14.8X	42.3	42.0	0.88		41.9	40.6	0.75
20.7X	42.8	41.3	0.88		42.4	39.4	0.75
28.9X	43.3	41.7	0.88		42.9	40.3	0.88

Table 3

Dilution	Error rate ORF1ab (%)	Error rate N (%)
2×	0.2	0.2
3×	0.2	0.2
4×	0.6	0.5
5×	2.6	1.5
6×	2.7	2.6
7×	3.5	3.2
8×	4.3	3.9
9×	5.0	4.4
10×	5.4	4.8
11×	6.4	5.8
12×	7.0	6.4
13×	7.6	7.0
14×	8.3	7.6
15×	9.0	8.2
16×	9.7	8.7

Patient Dataset

In this partition of the dataset, we had access to additional information for each sample, such as sex; age; date of onset of symptoms; date of the medical consultation; initial patient classification; information about the patients' occupation; affiliation to the health system; travels (international or domestic); comorbidities; symptoms; and if they had come into contact with a confirmed or suspected COVID-19 case. We collected 2,068 samples with an overall prevalence of 6.91% from April 18th to July 15th 2020. These samples correspond to 1,142 male patients (55%) and 926 (45%) female patients. The patients' ages ranged from 0 to 93 years, with a median age of 36 years. This additional information followed the protocol by the Colombian Public Health Surveillance System (SIVIGILA).

Test Center Dataset

We organized the second group of data according to the test center that collected the sample. We had access to information about the test centers, such as their location and name, but no information regarding the individual patients. This dataset included 7,162 samples, from 101 test centers, from April 6th to May 25th 2020 with a prevalence of 15.04%.

Training of the predictors

Dataset division

For the Patient Dataset, the distribution of epidemiological and social characteristics of individuals that get tested for COVID-19 can change drastically within a day. We cannot explicitly model the time variations in this dataset because we do not have the same patient at different times. Thus, the best way to exploit all of the specific information that we have from individuals is to create a two-fold cross-validation scheme, where we use one data fold for training and the held-out fold for evaluating the model. We created the folds using a stratified sampling strategy, where each fold had the same data distribution. However, notice that, even though we trained on a cross-validation scheme, our method can be evaluated in a real-life scenario where we perform daily predictions (see Section).

For the Test Center Dataset, we modeled the data according to each test center and its progression through time. The prevalence in the test centers evolves with the pandemic's development. We defined three temporal divisions of the data chronologically for our experiments: the training, validation, and test splits. For validation and testing, we held out five dates to predict in accordance with data availability. To obtain our final results in the test set, we retrained the best model with the training and validation samples, that is, those up to May 7th.

Training the models

We used the AutoML library from H2O³⁶ to explore multiple ML models and their hyperparameters in the validation sets, namely Random Decision Forests³⁷, Generalized Linear Models³⁸, Gradient Boosting Machines (GBM)³⁹, Fully Connected multi-layer Neural Networks⁴⁰, and Stacked Ensembles. Among the available models, we did not include deep learning algorithms because of the relatively small size of the datasets. We describe the modeling process for the *Patient Dataset* in the following section and detail the *Test Center Dataset*'s training process in the supplementary information

Patient Dataset. We trained our method to predict the label of a sample as positive or negative for COVID-19 by optimizing the Area under the Precision-Recall Curve. For these experiments, we defined a per-sample descriptor in which each feature dimension corresponds to a variable from the patient and location information, previously defined in section . The trained model's output is a probability for each patient to test positive. We obtained the final prediction via our cross-validation scheme by merging each split's prediction. For simplicity, in our main results, we used a pool size of 10, and we compare in the supplementary Fig. 1 this strategy and the optimal strategies per prevalence according to the findings from Section .

The best model for this task was a GBM³⁹ with 30 trees, a mean depth of 9, a minimum of 12 leaves, and a maximum of 22 leaves. We include a comparison of different models in the first section of the supplementary information.

Mathematical Formulation

Let $I = \{i_1, i_2, \dots, i_n\}$ be the set of n samples arriving to a testing facility, and $\mathcal{I}$ be space of all possible samples, $I \subset \mathcal{I}$. To enhance the performance of the Dorfman testing, Smart Pooling follows a similar approach of McMahan *et al.*'s *Optimal Dorfman with Threshold* procedure¹⁰. Let f be an ML model, $f : \mathcal{I} \rightarrow [0, 1]$, that computes the individual probability $p = f(i)$ of a sample $i \in \mathcal{I}$ from having the disease. Note that f is trained on a set $I_t \subset \mathcal{I}$ that does not intersect I , i.e. $I \cap I_t = \emptyset$. First, f extracts every probability $p_j = f(i_j)$ for all $i_j \in I$ and creates a new ordered set $I' = \{i'_1, i'_2, \dots, i'_n\}$, where $f(i'_j) \leq f(i'_{j+1})$, $\forall j \in \{1, 2, \dots, n-1\}$. Then, given a fixed pool size of c and a threshold T , we splitted I' into two subsets $I'_{<T} = \{i' | i' \in I' \text{ s.t. } f(i') < T\}$ and $I'_{\geq T} = \{i' | i' \in I' \text{ s.t. } f(i') \geq T\}$. The samples within $I'_{\geq T}$ are tested individually, and the ones belonging on $I'_{<T}$ are tested on a Dorfman's pooling fashion. For the latter, we splitted $I'_{<T}$ into $m = \lceil \frac{|I'_{<T}|}{c} \rceil$ groups, where $\lceil \cdot \rceil$ is the ceil operation. Let $I'_k = \{i'_j | i'_j \in I'_{<T} \text{ s.t. } (k-1)c + 1 \leq j \leq kc\}$, for $k \in \{1, 2, \dots, m\}$, be the set of the k 'th ordered set, with $\bigcup_{k=1}^m I'_k = I'_{<T}$. We followed the commonly used Dorfman's testing on these sets, including the consequent individual testing whether the group test was positive.

The efficiency of the aforementioned process is computed by counting the number of test kits used to generate the results divided by n . That is, the efficiency E of the set I , given a threshold T , a pool size c and an ML model f , is:

$$E(I, T, c, f) = \frac{1}{|I|} \left[|I'_{\geq T}| + \sum_{k=1}^m \left(1 + |I'_k| \mathbb{I}(I'_k) \right) \right] \quad (1)$$

where $\mathbb{I}(I'_k)$ is equal to one if at least one sample within I'_k is positive, and otherwise is zero. Thus, to determine T for the *Patient Dataset* we optimized the efficiency in the training folds. Given the models f_1 and f_2 trained on the folds I_{t1} and I_{t2} , respectively, the threshold is:

$$T_{\text{Patient}} = \underset{\mathcal{T} \in [0,1]}{\operatorname{argmin}} [E(I_{t1}, \mathcal{T}, c, f_2) + E(I_{t2}, \mathcal{T}, c, f_1)] \quad (2)$$

For the *Test Center Dataset*, to compute T we selected the optimal threshold on the validation set V , given the pool size c and a model f :

$$T_{\text{Test Center}} = \underset{\mathcal{T} \in [0,1]}{\operatorname{argmin}} [E(V, \mathcal{T}, c, f)] \quad (3)$$

Evaluation

We estimated the efficiency gains by calculating the expected number of tests per specimen. When this value is minimized, efficiency is maximized. To estimate the expected number of tests per specimen at different prevalences of the disease, we synthetically produced subsets of the dataset by randomly excluding negative samples. We performed 10 replicates of these experiments and computed the average and the standard deviation. For our experiments, we assumed a perfect sensitivity from the molecular tests. We refer the readers to the work of McMahan *et al.*¹⁰ for an analysis of the effects of reduced sensitivity on group testing efficiency.

Data Availability

The Test Center Dataset used during the current study is available at the [Smart Pooling repository](#). Patient Dataset belongs to a Colombian government entity and given the confidentiality agreements it is not publicly available.

Code Availability

The main code to reproduce our results is available at the [Smart Pooling repository](#). More information can be found at the [Smart Pooling website](#).

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Legends

Figure 1. Smart Pooling makes pooling methods efficient even at high disease prevalences.

a. In standard Dorfman's testing methods, samples are pooled randomly. As prevalence increases, the efficiency of pooling without a priori information drops rapidly, making the strategy unviable. **b.** Smart Pooling tackles this problem by thresholding samples according to the automated predicted risk probability, based on the sociodemographic characteristics. If the risk probability of a sample surpasses the defined threshold, this sample goes directly to individual testing. We arrange samples into pools with similar risk probability. **c.** The Smart Pooling pipeline directly integrates machine learning with laboratory procedures.

Figure 2. Smart Pooling decreases the expected number of tests per specimen.

a. Smart Pooling's expected number of tests per specimen compared to standard testing methods on Patient Dataset. Efficiency improves by reducing the expected number of tests per specimen. Smart Pooling achieves higher efficiencies than Dorfman's and individual testing for prevalences of the disease of up to 50%, with a fixed pool size of 10. **b.** Smart Pooling's expected number of tests per specimen trained with the coarse metadata from the Test Center Dataset. Efficiency improves by reducing the expected number of tests per specimen. Despite not having detailed patient metadata, Smart Pooling can produce higher efficiencies than Dorfman's pooling for the simulated prevalence of the disease of up to 25%, with a fixed pool size of 10. **c.** Expected number of tests per specimen for the proof-of-concept Smart Pooling Implementation. Each point on the graph represents the performance of both methods for a day in the proof-of-concept stage. The x-axis depicts the incidence of the corresponding day. Efficiency improves by reducing the expected number of tests per specimen. Smart Pooling has a similar efficiency compared to Dorfman's testing since the daily incidence is lower than 10%. However, for every day of the implementation, Smart Pooling has a higher efficiency than individual testing.

Figure 3. Comparison between Smart Pooling and Random ordering.

Left: samples arranged by Smart Pooling. **Right:** random ordering of samples. Note that Smart Pooling groups positive samples generating long groups of negative samples, reducing the number of times it is necessary to perform the second stage of Dorfman's testing, increasing the efficiency.

Figure 4. Efficiency of two-stage Dorfman's pooling as a function of prevalence p and pool size c

Figure 5. Optimal pooling strategies given a maximum pool size of a. 10 and b. 5.

Table 1. Optimal pooling strategies restricted by a maximum pool size c .

Table 2. Expected C_t , average C_t , and the fraction of wells with no reading for markers N and ORF1ab for successive dilutions of the same sample.

Table 3. Estimated upper bound for the error (false negative) rate for single pools of up to 16 samples for markers N and ORF1ab.

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Author contributions statement

ME, GJ, LBS, AC, CG, DV, MFR, and JM designed and trained the AI models, and curated the dataset. MC, MGS, JMPL, and SR performed the molecular tests. ALM, MFS, MV, and JMPL developed mathematical formulations to find the optimal pooling strategies. MFS and JMPL evaluated the applicability of the testing protocols in the laboratory. ALM, JMPL, MV and PA developed the theoretical interpretation of the results. LBS, GJ, JMW, JM, MFR, ALM, and MV prepared the figures. ME, GJ, LBS, AC, CG, DV, MFR, JM, JMW, ALM, MFS, and MV wrote the manuscript. All authors revised and accepted the final version of the manuscript. SR and PA supervised and directed the research.

Competing interests

The authors declare no competing interests.

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