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Nasal or throat swabs from several people can be combined in a single test.

THE MATHEMATICAL STRATEGY THAT COULD TRANSFORM CORONAVIRUS TESTING

To save time and money, several countries are using a technique called group testing, which pools samples from many people.

By Smriti Mallapaty

Scientists say that widespread testing is needed to get outbreaks of the new coronavirus under control. But, in many regions, there's a shortage of the chemicals needed to run diagnostics. Researchers are scrambling to devise faster, simpler tests (see page 506). Now, in several countries, officials have started using a strategy that was first proposed in the Second World War: group testing. By pooling samples from many people, this method can save time, chemical reagents and money, say researchers.

"In the current epidemic, there is a need to test an extremely large number of patients, making pooling an attractive option," says Roy Kishony, a systems biologist at Technion – Israel Institute of Technology in Haifa. China, India, Germany and the United States are already using group testing.

There are many ways to conduct group testing, and scientists in several countries are experimenting with the best method for doing this during a pandemic. Their ideas largely come from a field of mathematics that has been applied to a wide range of problems, from detecting faulty Christmas-tree lights to estimating the prevalence of HIV in a population. "There has been a flurry of innovation in this field," says Dror Baron, an information scientist at North Carolina State University in Raleigh.

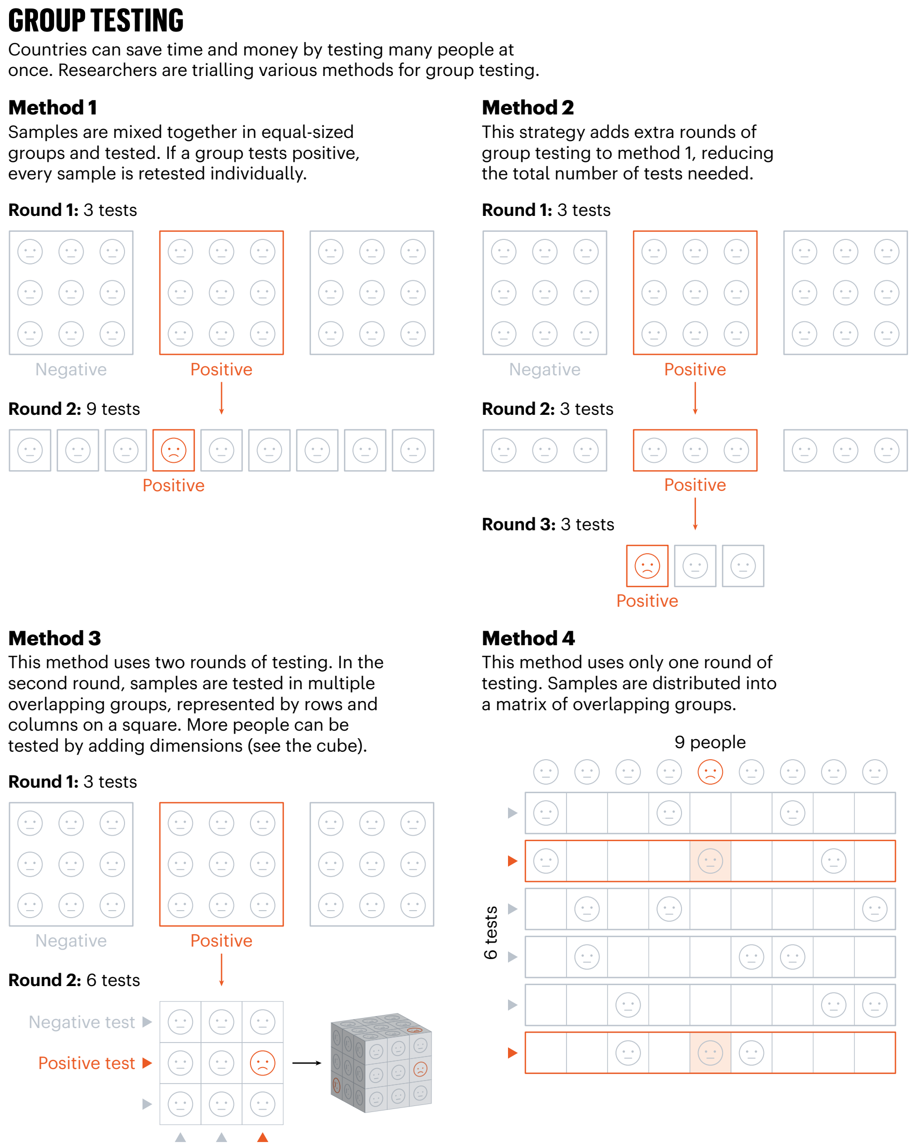
Started with syphilis: Methods 1 & 2

The simplest group-testing strategy was proposed by economist Robert Dorfman in the 1940s to test soldiers for syphilis.

In this method, an equal number of samples – collected from nasal and throat swabs in the case of the SARS-CoV-2 virus – are mixed together and tested once (see 'Group testing', Method 1). Groups of samples that test negative are ruled out. But if a group tests positive, every sample in that group is then retested individually. Researchers estimate the most efficient group size – the one that uses the least number of tests – on the basis of the prevalence of the virus in the community.

In May, officials in Wuhan, China, used Method 1 as part of their efforts to test the vast majority of the city's population, reaching roughly ten million people in just over two weeks. Samples from some 2.3 million people were group tested, with up to 5 samples in a group, and 56 infected people were identified.

The method is most efficient when there are low levels of infection, in around 1% of the population, because group tests are more likely to be negative, which saves testing many people individually, say researchers.



SOURCES: METHOD 1: R. DORFMAN *ANN. MATH. STATIST.* **14**, 436–440 (1943); METHOD 3: L. MUTESA, *ET AL.* PREPRINT AT HTTPS://ARXIV.ORG/ABS/2004.14934 (2020); METHOD 4: MANOJ GOPALKRISHNAN

"This is probably the easiest method," says Krishna Narayanan, an information theorist at Texas A&M University in College Station. But there are more efficient ways to construct the second stage than testing everyone individually, he says.

A more sophisticated version involves adding more rounds of group tests, before testing each sample separately (see 'Group testing', Method 2). Adding rounds reduces the number of people who need to be tested individually.

But this approach means waiting several hours to get results for each group test, says Wilfred Ndifon, a theoretical biologist at the African Institute for Mathematical Sciences in Kigali, Rwanda. "This is a fast-growing, fast-spreading disease. We need answers much faster than this approach would allow," he says.

Multi-dimensions: Method 3

Ndifon and his colleagues have improved on Dorfman's strategy, and are planning a trial in Rwanda that should ultimately reduce the number of tests needed. Their first round of group tests is the same as Dorfman's, but for groups that test positive, they propose a second round that divides samples between groups that overlap.

Imagine a square matrix with nine units, each representing swabs taken from one person (see 'Group testing', Method 3). The samples in each row are tested as one group, and the samples in each column are tested as one group, resulting in six tests in total, with each person's sample being in two groups. If a sample contains SARS-CoV-2 viral RNA, both of the group tests will be positive, making it easy to identify the person. Researchers describe the idea in a preprint posted on the arXiv server on 30 April (L. Mutesa *et al.* Preprint at https://arxiv.org/abs/2004.14934; 2020).

Increasing the number of dimensions, for example from a square to a cube, allows for larger group sizes and higher gains in efficiency, says Neil Turok, a theoretical physicist at the University of Edinburgh, UK, and a study co-author.

Ndifon, who is part of Rwanda's COVID-19 task force, says group testing is part of the government's strategy to quickly identify and isolate infected people. He and his colleagues estimate that their method could cut the cost of testing from US$9 per person to 75 cents. The researchers are carrying out laboratory experiments to see how many samples can practically be included in a group test and still detect a positive result. Co-author Leon Mutesa, a geneticist at the University of Rwanda in Kigali, who is also part of the government task force, says that he has identified one positive sample in a pool of 100 in the lab.

But Sigrun Smola, a molecular virologist at Saarland University Medical Center in Homburg, Germany, who has been testing samples in groups of up to 20, doesn't recommend grouping more than 30 samples in one test, to ensure sufficient accuracy. Larger groups will make it harder to detect the virus, and increase the chances of missing positives, she says. Smola is also sceptical of the practical application of the cube-slicing technique. "If you told this to a technician, they would say, 'What a mess. I want a simple scheme,'" she adds.

Ndifon says that his team plans to develop software to automate sample placement.

One-step solution: Method 4

Some researchers say that even two rounds of testing is too many when trying to curb a fast-spreading virus such as SARS-CoV-2. Lab technicians must wait for the results from the first round to come through, which slows the process, says Manoj Gopalkrishnan, a computer scientist at the Indian Institute of Technology Bombay in Mumbai.

Instead, Gopalkrishnan proposes doing all the tests in one round, with many overlapping groups. This would increase the number of tests, but would save time — although the initial set-up is time-consuming, because extra groups means more samples must be pipetted.

Gopalkrishnan's approach involves mixing samples in different groups, using a counting technique known as Kirkman triples, which sets rules for how the samples should be distributed. Imagine a flat matrix in which each row represents one test, and each column represents one person (see 'Group testing', Method 4). Generally, every test should include the same number of samples, and each person's sample should be tested the same number of times.

But Narayanan says that one-step strategies require more tests to ensure a similar accuracy to that of multi-step group testing. One-step approaches also mean working with many samples at once, which can be tricky, he says.

To simplify the process, Gopalkrishnan and his colleagues have developed a smartphone app that tells users how to mix the samples. In unpublished results from clinical trials in India in Mumbai, Bengaluru and Thalassery, he says, 5 positive samples were successfully identified out of 320, using only 48 tests.