Multiplying testing capacity
RT PCR Pool Testing

Pilot Testing at ELEAM Care Facilities

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Summary

• We propose the use of a PCR Pool Test for preventive testing (screening), starting with a pilot test in long-term care establishments for senior citizens.

• Idea: Early identification of Covid-19 cases (vulnerable members of the population and/or medical staff).

• Pool Testing: samples from several people (5 to 10) analyzed with one PCR kit.

• If the test is negative, it is declared negative for all.

• Use: Israel and some areas in the USA. Clinical trial in Chile at the Universidad de Chile.

• The protocol does not affect the usual PCR technique.

• Group testing allows us to: (i) multiply several-fold the testing capacity, (ii) deliver test results quicker.

• If escalation continues after testing: support for ISCI logistics.
Background

• Probability of detecting the virus during the chain of infection, for different types of test.

• Screening vs. Diagnosis vs. Incidences.

• PCR useful for detection during the infection stage (and afterwards).

Background

• Objective: Mass PCR screening.

• Barriers:
  • Logistics: sample collection, laboratory resources, etc.
  • Limited resources: nasal swabs, reagents, PCR/laboratory machines, personnel, transport, etc.
  • Testing capacity: currently limited by laboratory components (PCR machines, reagents, etc.)
  • Delay in the delivery of results.

**Solution:** Group testing to minimize the use of scarce resources and to reduce delivery times for results.
Background

• Individual paradigm testing:
  • One test per sample.
  • \(N\) tests required to test \(N\) samples.
Background

• Group paradigm testing:
  • **Multiple** samples are combined into **one single test**.
Group Testing

• **Negative result:** Each of the simples would have delivered a negative result under the paradigm of individual testing.

• **Positive result:** At least one of the samples would have delivered a positive result under the paradigm of individual testing.

When the prevalence of the virus is low in the population, it is possible to test groups of people with a single test (saving resources and time).
2-Stage Testing Policy

- Proposal by Dorfman (USA, 1944) to identify recruits with syphilis through the analysis of blood samples.
  - Stage I:
    - Combined sample is tested. If the result is negative, it is concluded that there are no recruits with syphilis.
  - Stage II:
    - If there is a pool that is positive, each recruit in the pool is tested individually.

When the prevalence is low, advance to stage II with low frequency. This permits the use of fewer kits.
2-Stage Testing Policy

• The efficiency of the method depends on the size of the group (pool) and the prevalence of the virus.

• How many tests are needed on average to test 1,000 people?
  • \( p = 0.1 \Rightarrow \text{tests} \approx 600 \)
  • \( p = 0.05 \Rightarrow \text{tests} \approx 400 \)
  • \( p = 0.02 \Rightarrow \text{tests} \approx 280 \)
### 2-Stage Testing Policy

- **How many tests are required on average to test 1,000 people?**

<table>
<thead>
<tr>
<th>Incidences</th>
<th>Pool Size</th>
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<td>2%</td>
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<tr>
<td>5%</td>
<td>1000 598 476 435 <strong>426</strong> 432 445 462 481 501 543 603</td>
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<tr>
<td>8%</td>
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<td>9%</td>
<td>1000 672 580 564 576 599 626 655 683 711 761 824</td>
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<td>1000 690 604 594 <strong>610</strong> 635 665 695 724 751 801 861</td>
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<td>11%</td>
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Shorter Delivery Times of Results

Example
• Suppose it takes 5 hours for a laboratory to process each PCR.
• The aim is to test a group of 100 people.
• If using individual PCRs, it will take 500 hours to process the samples.
• Without pooling, the time it will take to have the test results of that population is 500 hours.

• If pools of 5 people are used and we assume a prevalence of 5%, 43 tests will be used on average, considering that a few pools will test positive, and it will be necessary to test individually (see table of use of tests according to prevalence).
• With PCR pooling, the time to have the test results of that population is 215 hours.

• The lower the prevalence in the population, the more time decreases. The time saving can be observed in the table.
• Additionally, the processing time is not constant. It increases with a greater flow of tests, which generates more time savings.
Covid-19 Context Background

- Yelin et al (Clin. Inf. Dis. 2020) – Validate group testing for groups of up to 32 without altering individual protocol, and 64 extending the cycle times.
- Nebraska reports implementation of the method in the public healthcare system (groups of 5, mid-March).
- Experience in Chile: Method validated for groups of 5 (to be examined shortly).
Challenges

- Group testing efficiency as a function of prevalence *(unknown)*.
  - Re-evaluate group size depending on the target population (eg. asymptomatic) and learning.
  - Validation method, protocol for laboratories.

- Possible bottlenecks further on.
  - Logistics of a mass testing program.
  - Testing frequency, quarantine policy, etc.
  - Increased efficiency via multi-stage testing (complexity!)
Clinical Trials: Pool Testing in Chile
Experiment carried out at the Center of Molecular Studies of the Faculty of Medicine, Universidad de Chile - HLCM.

Experiment replicated at the Virology Laboratory (Dr. F. Valiente – Dr. Soto-Riffo), ICBM, and Laboratory Universidad de Magallanes.

Considered % of positivity: 8-10%
23 positive samples (Ct: 16-36)
40 negative samples
Optimizing RT-PCR detection of SARS-CoV-2 for developing countries using pool testing

Muestra nasofaríngea (n=5)

200 μL cada uno

1 mL

RT-PCR SARS-CoV-2
(High Pure Viral NA Kit - Roche®)

5 μL

Extracción Automatizada
(MagnaPure - Roche®)

Extracción Manual
(High Pure Viral NA Kit - Roche®)

Farfán M, Torres JP, O’Ryan M, Olivares M, et al (Frontiers in Cellular @ Infection Microbiology (en rev.))
Optimizing RT-PCR detection of SARS-CoV-2 for developing countries using pool testing


Muestra nasofaríngea (n=5)

200 µL cada uno

1 mL

MUT

RT-PCR SARS-CoV-2
(High Pure Viral NA Kit - Roche®)

5 µL

Extracción Automatizada
(MagnaPure - Roche®)

Extracción Manual
(High Pure Viral NA Kit - Roche®)
Figure 1. Amplification curves of SARS-CoV-2 obtained for pool 9. SARS-CoV-2 RT-PCR was done using as template nucleic acids purified from automated and manual extraction, or the pool sample (no extraction). NTC, no template control.
Optimizing RT-PCR detection of SARS-CoV-2

Table 1. SARS-CoV2 PCR results obtained from the first six pools of nasopharyngeal samples. Nucleic acids extraction was performed using an automated extraction.

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Optimizing RT-PCR detection of SARS-CoV-2

Table 2. SARS-CoV-2 PCR results obtained from 5 pools of nasopharyngeal samples. Nucleic acids extraction was performed using an automated \(^a\) (A) and manual \(^b\) (M) extraction. Adding pool sample (P) directly to PCR reaction was also evaluated \(^c\).

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Figure 2. \( C_T \) values of amplification results of SARS-CoV-2 for pools 12-31. SARS-CoV-2 RT-PCR was done using as template nucleic acids purified from manual extraction and the \( C_T \) values obtained in the single positive samples (blue dots) and its respective pool (red dots) were graphed. Also, the change in \( C_T \) value compared with \( C_T \) value of the single positive sample present in the pool is shown in brackets. A \( C_T \) value of 0 was assigned to samples with no amplification. Pool 26 is highlighted (black arrow).
Testing phase trials via pooling in long-term care facilities for senior citizens (ELEAM)
Preventive Testing Trials at ELEAM Facilities

- Plan to reduce rate of infections at ELEAM facilities - Prevention, testing, and isolation.
  - National Service for Senior Citizens (SENAMA) and Chilean Association of Safety (ACHS).

- AChS, the Faculty of Medicine of the University of Chile and the Complex Engineering Systems Institute propose:
  - The use of PCR pooling techniques for preventive testing in ELEAM facilities.
  - This allows early identification of COVID-19 infections and reporting in less time than if individual tests were used.
  - Prevents quickly, efficiently, and continuously the advance of the virus among more vulnerable members of the population.
Preventive Testing Trials at ELEAM Facilities

• Progressive implementation - Trial
  • AChS, with the backing of the Ministry for Health and SENAMA, takes samples in one or more ELEAM facilities.
  • Samples tested with pooling techniques by the Universidad de Chile.
  • Results transmitted to the corresponding Health Service.

• Scaling
  • Transfer of protocols from UCH to other laboratories.
  • Analysis of other pool sizes-
  • ISCI advice on all issues that will emerge from logistics, dispatch, and sample matching laboratories.
  • Other applications (Hospital de Ñuble, Hospital del Trabajador).