Multiplying testing capacity
RT PCR Pool Testing

Pilot Testing at ELEAM Care Facilities

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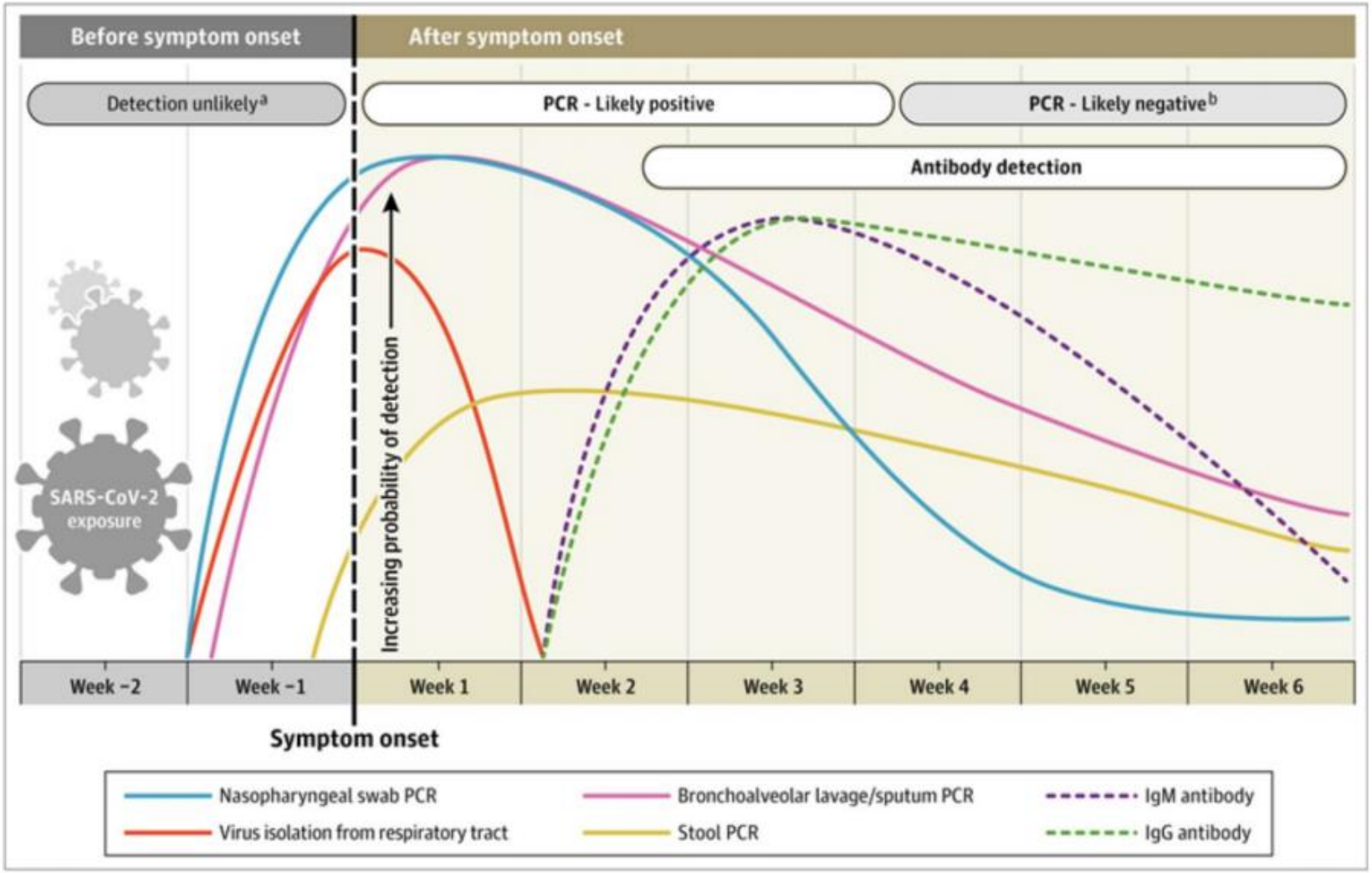
Summary

- 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).



Source: "Interpreting Diagnostic Tests for SARS-CoV-2", Sethuraman et al, JAMA, 2020.

Background

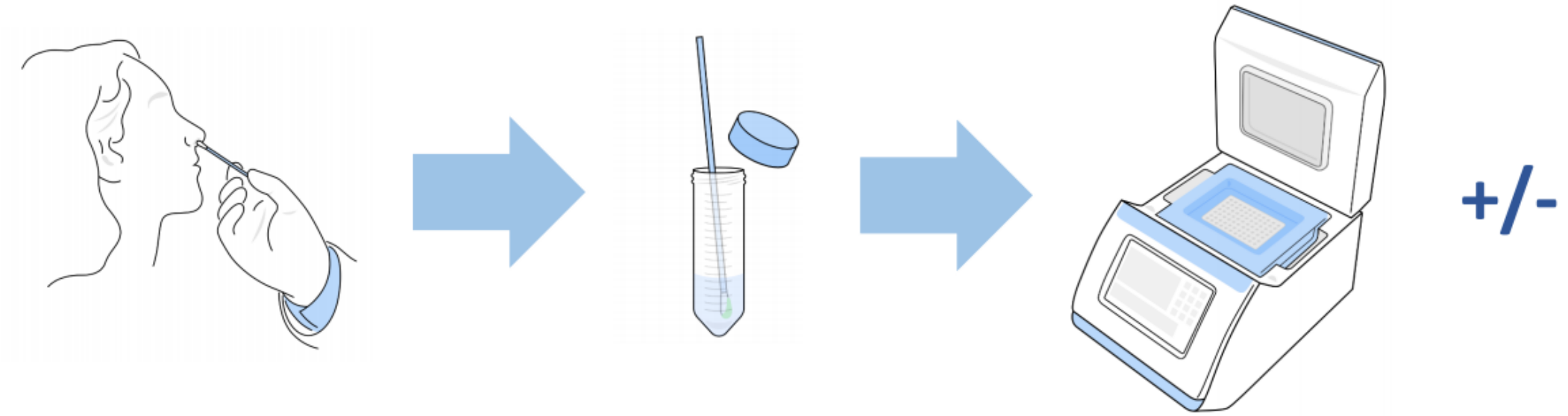
- Objective: Mass PCR screening.

- 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.

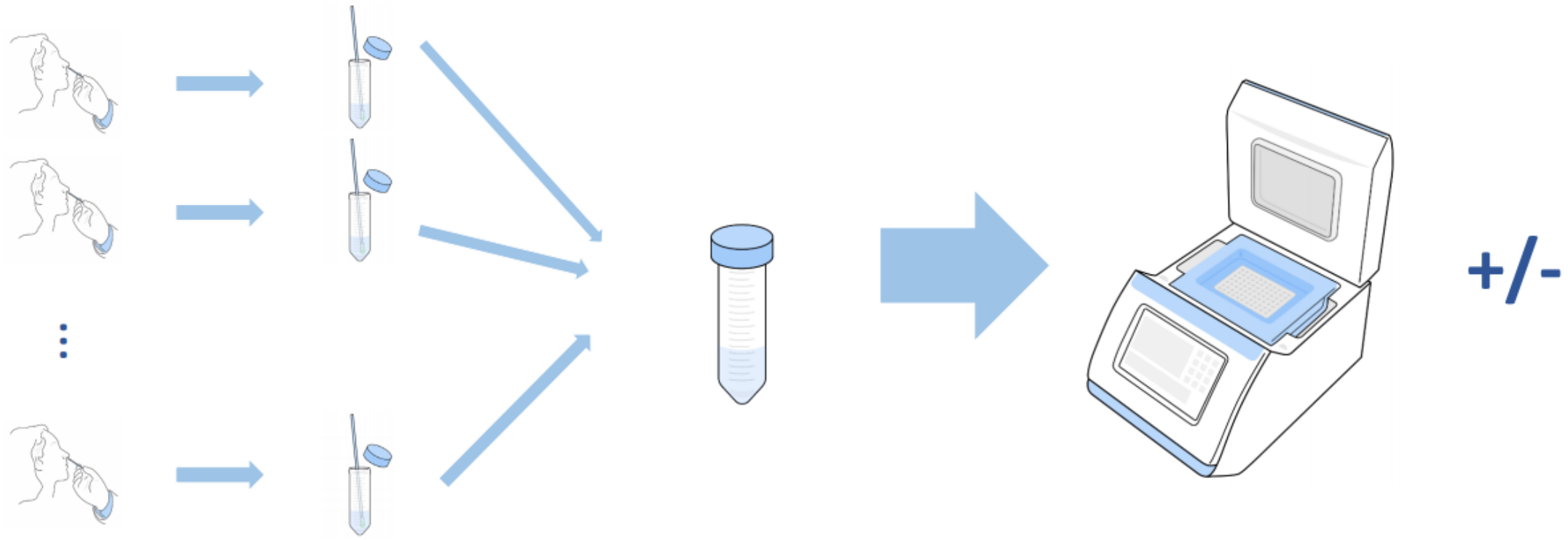
Background

- Individual paradigm testing:
  - One test per sample.
  - **N** tests required to test **N** samples.



Background

- Group paradigm testing:
  - **<u>Multiple</u>** samples are combined into **<u>one single test</u>**.



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

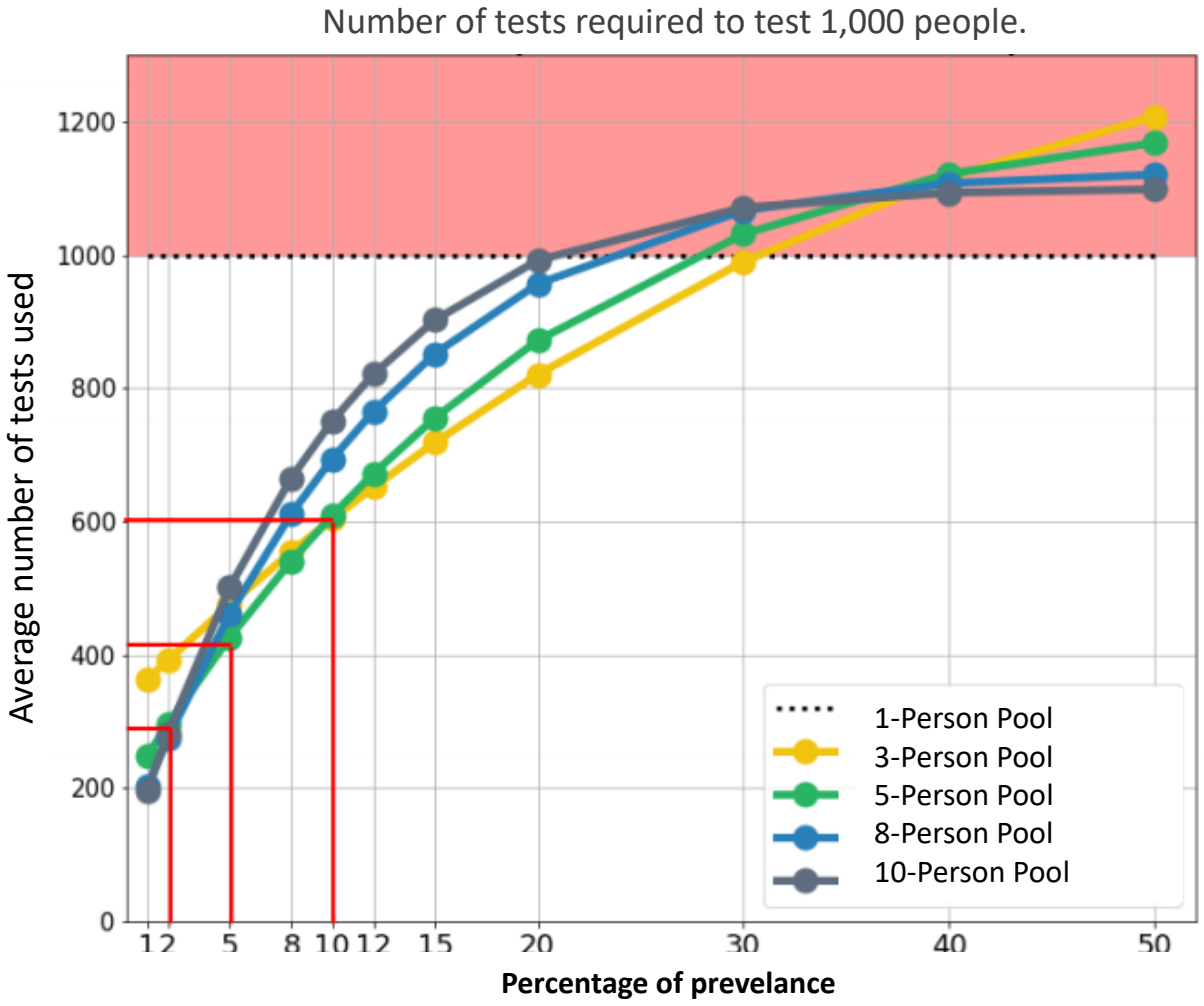
- **Proposal by Dorfman (USA, 1944) to identify recruits with syphilis through the analysis of blood samples.**
  - Stage I:
    - Combined sample is tested. If the result is negative, it is concluded that there are no recruits with syphilis.
  - Stage II:
    - If there is a pool that is positive, each 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 $\Longrightarrow$ tests ≈ 600
  - p = 0.05 $\Longrightarrow$ tests ≈ 400
  - p = 0.02 $\Longrightarrow$ tests ≈ 280



2-Stage Testing Policy

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

Incidences	Pool Size 1	2	3	4	5	6	7	8	9	10	12	15
1%	1000	520	363	289	249	225	211	202	198	196	197	207
2%	1000	540	392	328	296	281	275	274	277	283	299	328
5%	1000	598	476	435	426	432	445	462	481	501	543	603
8%	1000	654	555	534	541	560	585	612	639	666	716	780
9%	1000	672	580	564	576	599	626	655	683	711	761	824
10%	1000	690	604	594	610	635	665	695	724	751	801	861
11%	1000	708	628	623	642	670	701	731	761	788	836	893
12%	1000	726	652	650	672	702	734	765	795	821	868	920
15%	1000	778	719	728	756	790	822	853	879	903	941	979
20%	1000	860	821	840	872	905	933	957	977	993	1015	1031
30%	1000	1010	990	1010	1032	1049	1061	1067	1071	1072	1069	1062
40%	1000	1140	1117	1120	1122	1120	1115	1108	1101	1094	1081	1066
50%	1000	1250	1208	1188	1169	1151	1135	1121	1109	1099	1083	1067

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

Pool Testing RT-PCR SARS-CoV-2 in Chile

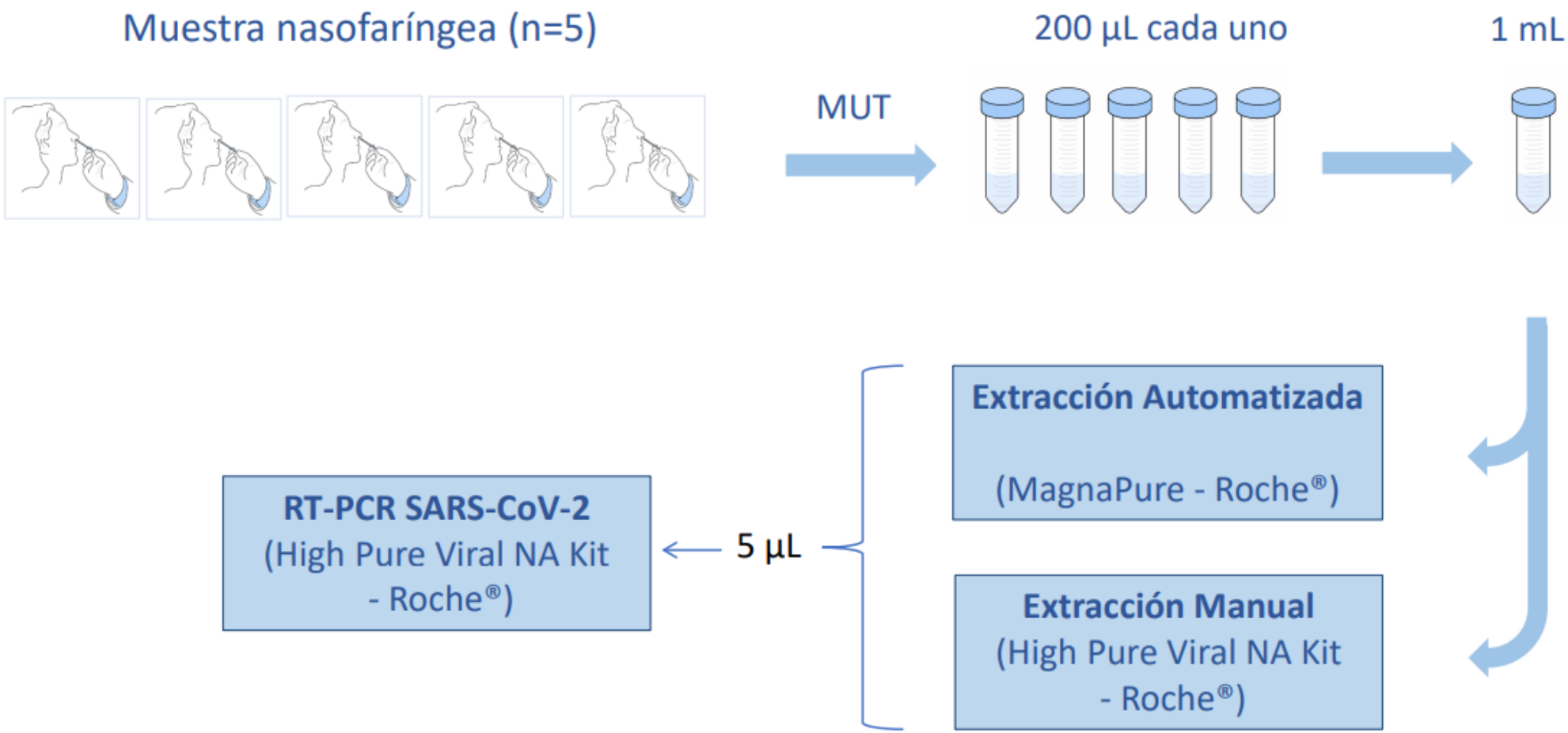
- Experiment carried out at the Center of Molecular Studies of the Faculty of Medicine, Universidad de Chile - HLCM.

- 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

Farfán M, Torres JP, O'Ryan M, Olivares M, et al (Frontiers in Cellular @ Infection Microbiology (en rev.)



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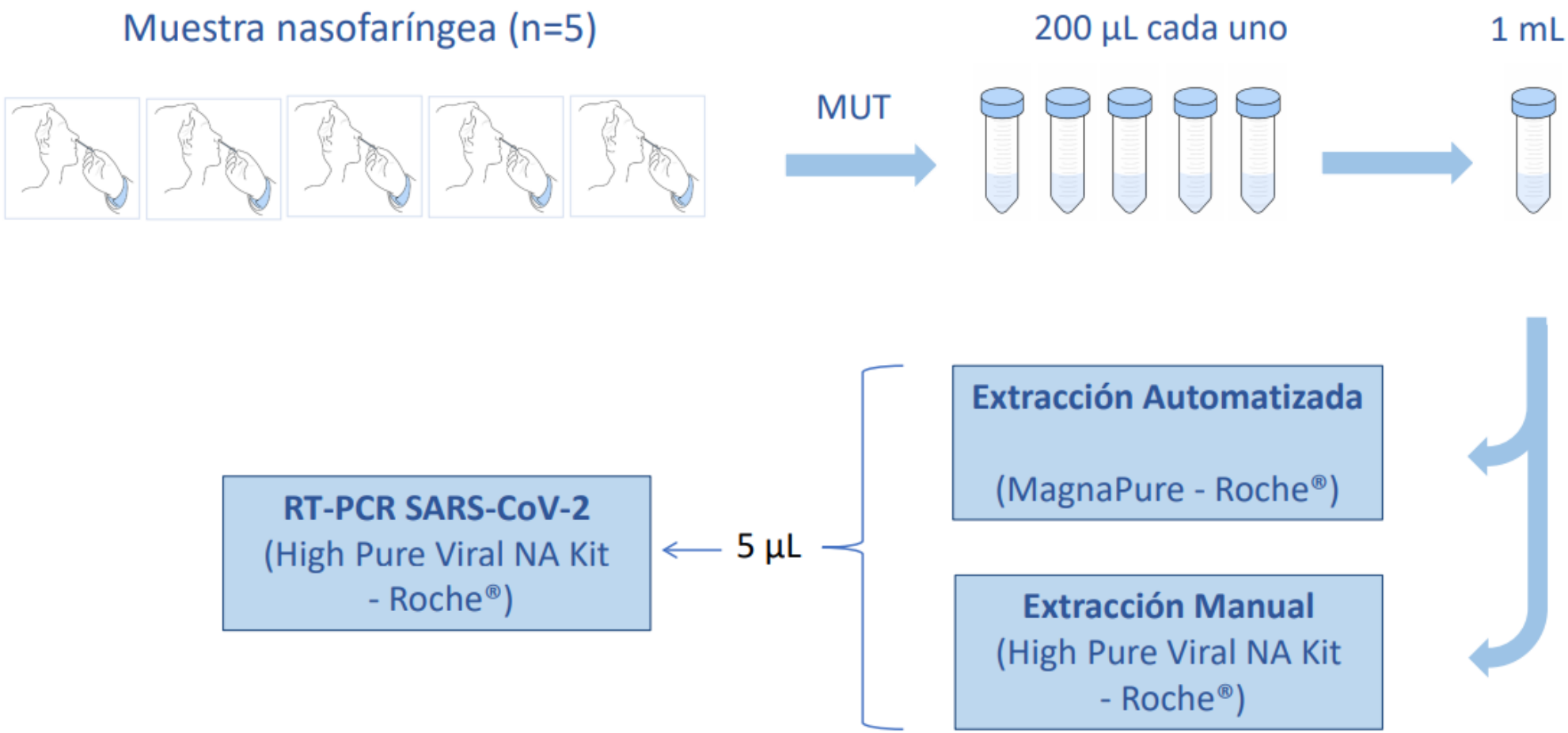

Optimizing RT-PCR detection of SARS-CoV-2

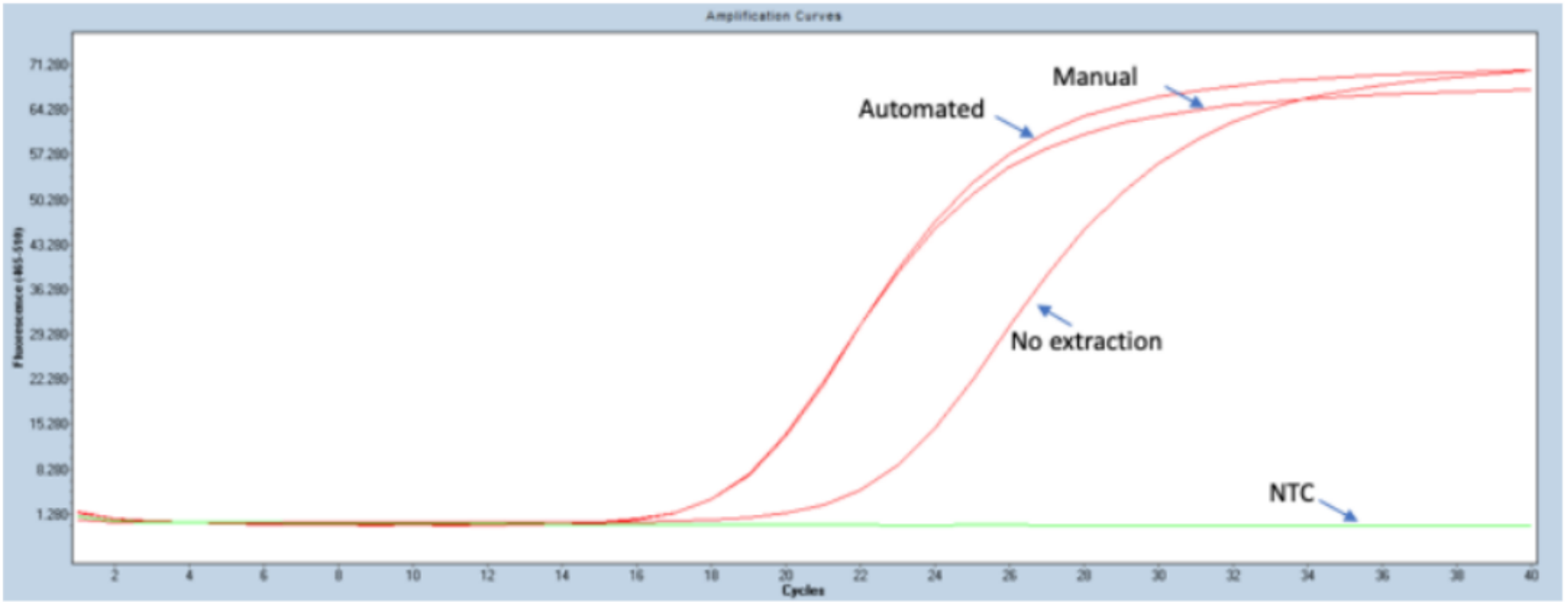

Figure 1. Amplification curves of SARS-CoV-2 obtained for pool 9. SARS-CoV-2 RT-PCR was done using as template nucleic acids purified from automated and manual extraction, or the pool sample (no extraction). NTC, no template control.

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 [a]

Sample	C_T Value SARS CoV-2	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
1	Neg	X	X				X
2	Neg	X	X				X
3	Neg	X	X				X
4	Neg	X	X			X	
5	Neg	X		X	X		
6	Neg			X	X	X	
7	Neg			X	X	X	X
8	Neg			X	X	X	
9	Neg			X			
10	21.1		X				
11	23.8				X		
12	26.9					X	
13	31.6						X
SARS-CoV-2 RT-PCR		Neg	Pos	Neg	Pos	Pos	Pos
C_T value		-	24.3	-	27.2	30.1	34.0
ΔC_T		-	3.2	-	3.4	3.2	2.4

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].

Sample	C_T Value SARS CoV-2	Pool 7A	Pool 8A	Pool 9A	Pool 10A	Pool 11A	Pool 7M	Pool 8M	Pool 9M	Pool 10M	Pool 11M	Pool 7P	Pool 8P	Pool 9P	Pool 10P	Pool 11P
14	Neg	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
15	Neg	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
16	Neg	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
17	Neg	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
18	Neg	X					X					X				
19	23,5		X					X					X			
20	16,8			X					X					X		
21	26,8				X					X					X	
22	35					X					X					X
SARS-CoV-2 RT-PCR		Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Neg
C_T		-	25.6	18.3	29.0	-	-	25.2	18.5	29.0	-	-	28.1	22.3	32.1	-
ΔC_T		-	2.1	1.5	2.2	-	-	1.7	1.7	2.2	-	-	4.6	5.5	5.3	-

Optimizing RT-PCR detection of SARS-CoV-2

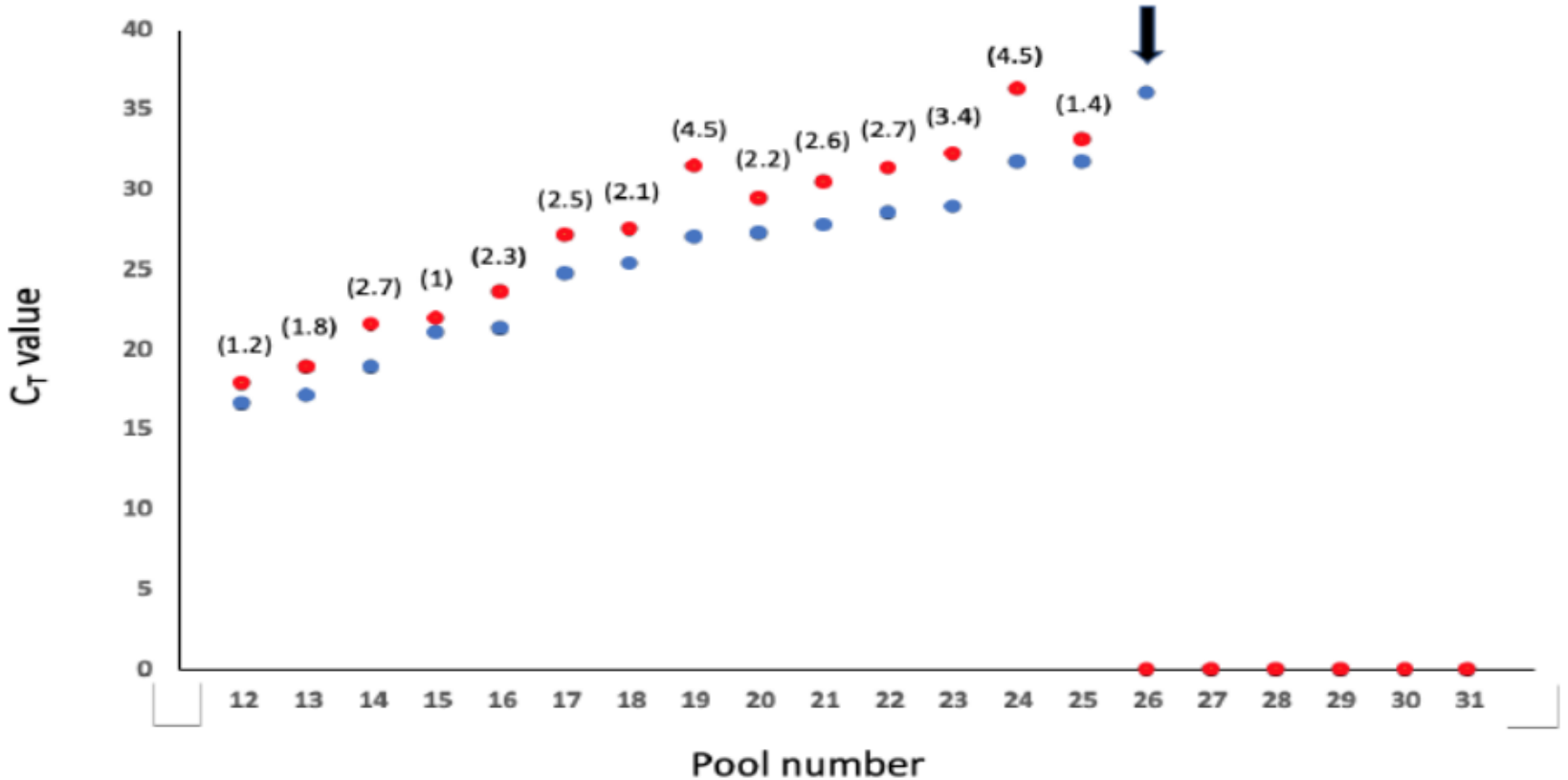

Figure 2. C_T values of amplification results of SARS-CoV-2 for pools 12-31. SARS-CoV-2 RT-PCR was done using as template nucleic acids purified from manual extraction and the C_T values obtained in the single positive samples (blue dots) and its respective pool (red dots) were graphed. Also, the change 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).

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