Instituto de Salud Pública
Ministerio de Salud

" Importancia e impacto de las mediciones de microvolúmenes en los ensayos clínicos“

"*Importance and impact of microvolume measurements in clinical assay*"

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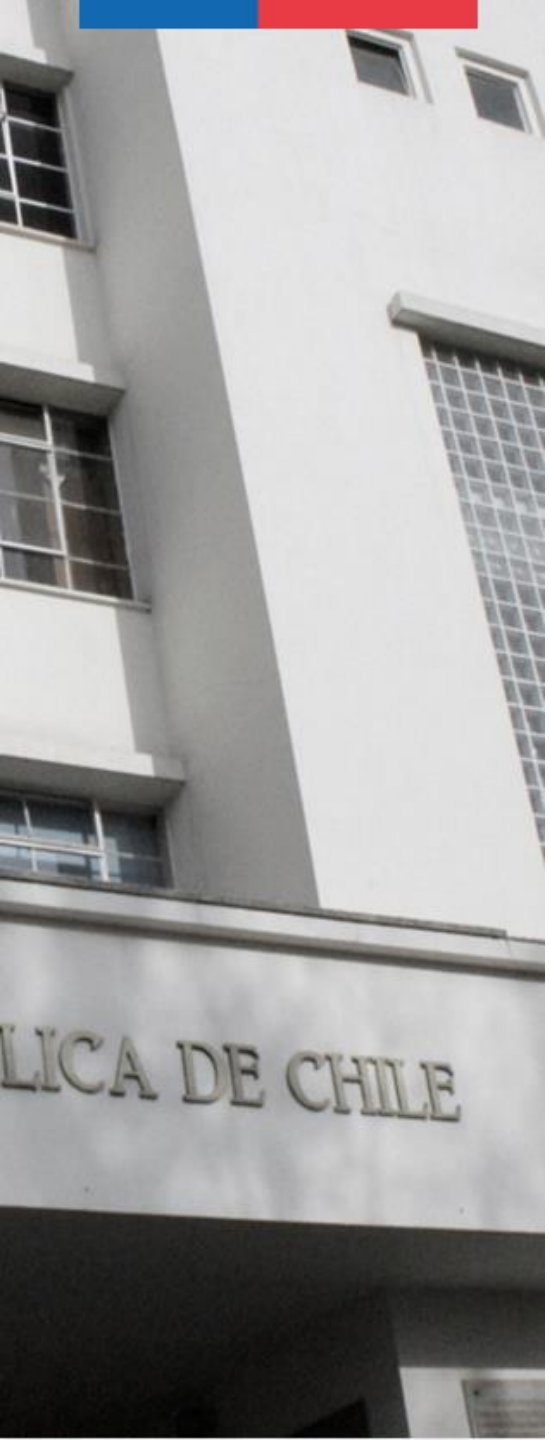
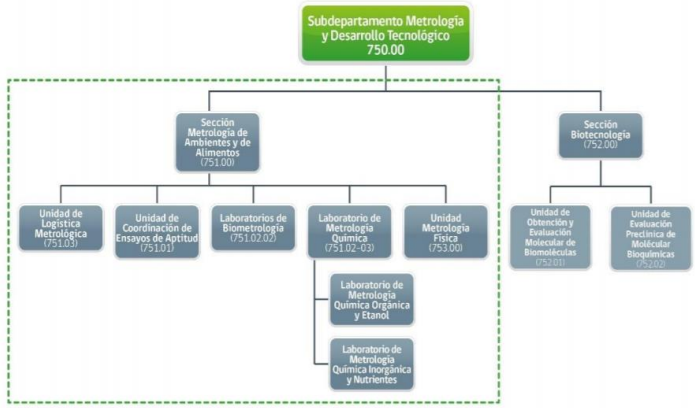


About us

The current Institute of Public Health of Chile (ISP) traces its historical roots to 1892 when, on September 15 of that year, the Institute of Hygiene was created by Law, under the direction of Dr. Federico Puga Borne.

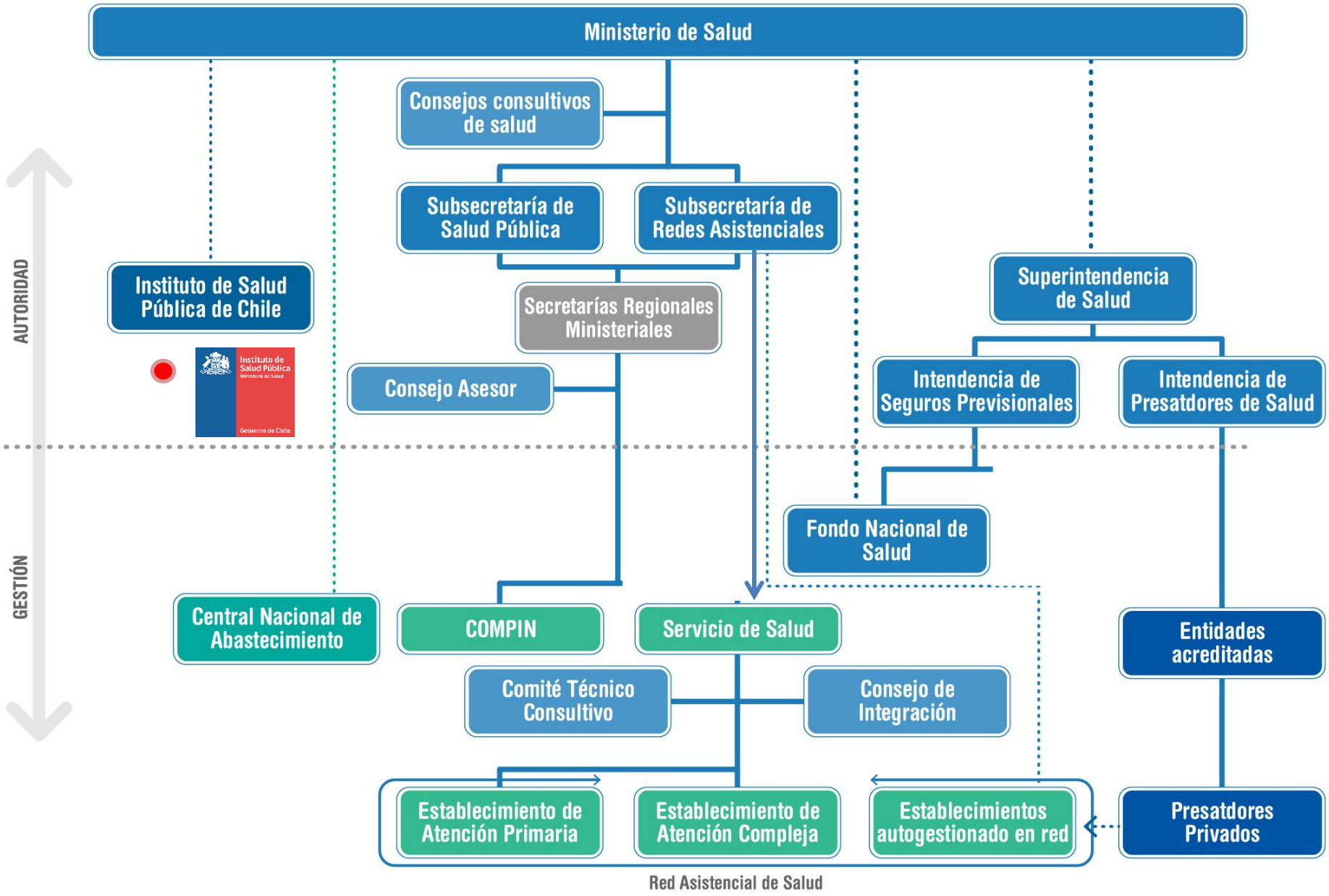
The Chilean Public Health Institute has the role of serving as a National and Reference Laboratory. It is an autonomous public service, it depends on the Ministry of Health for the approval of its policies, norms and general plans of activities, as well as for the supervision of their execution.

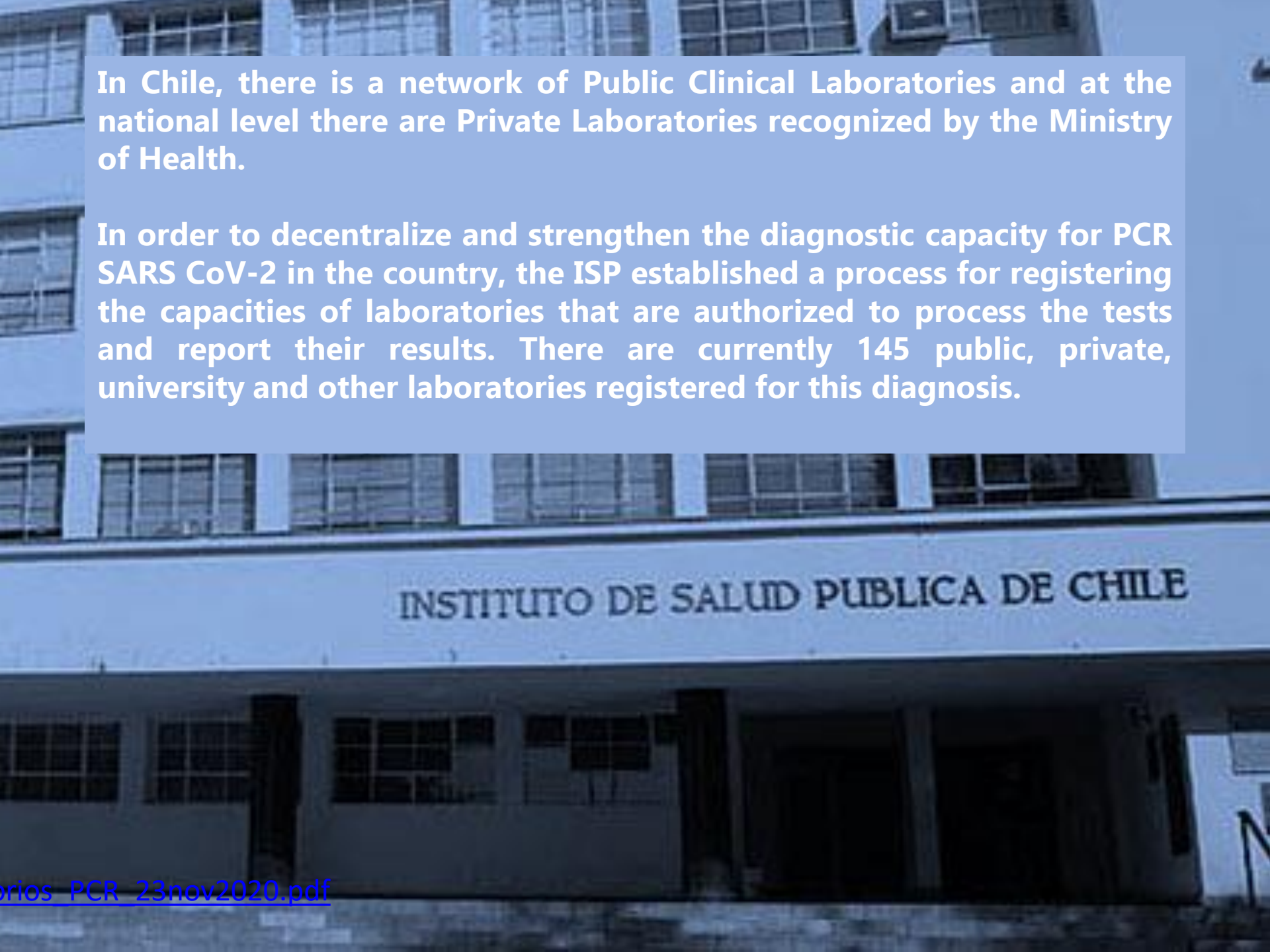
Organizacional Estructure



Nota 1: - - - Laboratorio Designado de la Red Nacional de Metrología

Chilean Health System





In Chile, there is a network of Public Clinical Laboratories and at the national level there are Private Laboratories recognized by the Ministry of Health.

In order to decentralize and strengthen the diagnostic capacity for PCR SARS CoV-2 in the country, the ISP established a process for registering the capacities of laboratories that are authorized to process the tests and report their results. There are currently 145 public, private, university and other laboratories registered for this diagnosis.

orios_PCR_23nov2020.pdf

Clinical Laboratory Net - COVID-19 in Chile

Fuente: www.minsal.cl www.minciencia.gob.cl

ISO 15189: 2012

Medical laboratories - Requirements for quality and competence

Clinical laboratories meet the quality requirements established in ISO 15189. This standard addresses management requirements and technical requirements.

Within the technical requirements it is considered: 5.3 Laboratory equipment, reagents and consumables, In 5.3.1.2. :

"The laboratory must verify after installation and before use that the equipment is capable of to achieve the necessary performance and to meet the relevant requirements of the examinations in question"

“ 5.3.1.4 Calibration and metrological traceability of equipment

The laboratory must have a documented procedure for the calibration of equipment that affects directly or indirectly the results of the exams. This procedure includes:

a) take into account the conditions of use and the manufacturer's instructions;
b) record the metrological traceability of the calibration standard and the traceable calibration of the equipment;
c) verify the required measurement accuracy and the operation of the measurement system at intervals defined;
d) record the status of calibration and recalibration date;
e) ensure that, when the calibration results in a set of correction factors, the factors previous calibration files are updated correctly;
f) security measures to prevent manipulation or adjustments that could invalidate the results of exams.”

Metrological traceability must be to a reference material or procedure of the highest order metrological available.

"5.3.1.5 Maintenance and repair of equipment:

…..Whenever equipment is found to be defective, it must be taken out of service and clearly labeled.

The laboratory must ensure that defective equipment is not used until it has been repaired and It is demonstrated by verification that it meets the specified acceptance criteria."

Among the various equipment used by laboratories are automatic pipettes, which allow the extraction of samples of liquid substances and reagents for the purposes of the clinical trials carried out.

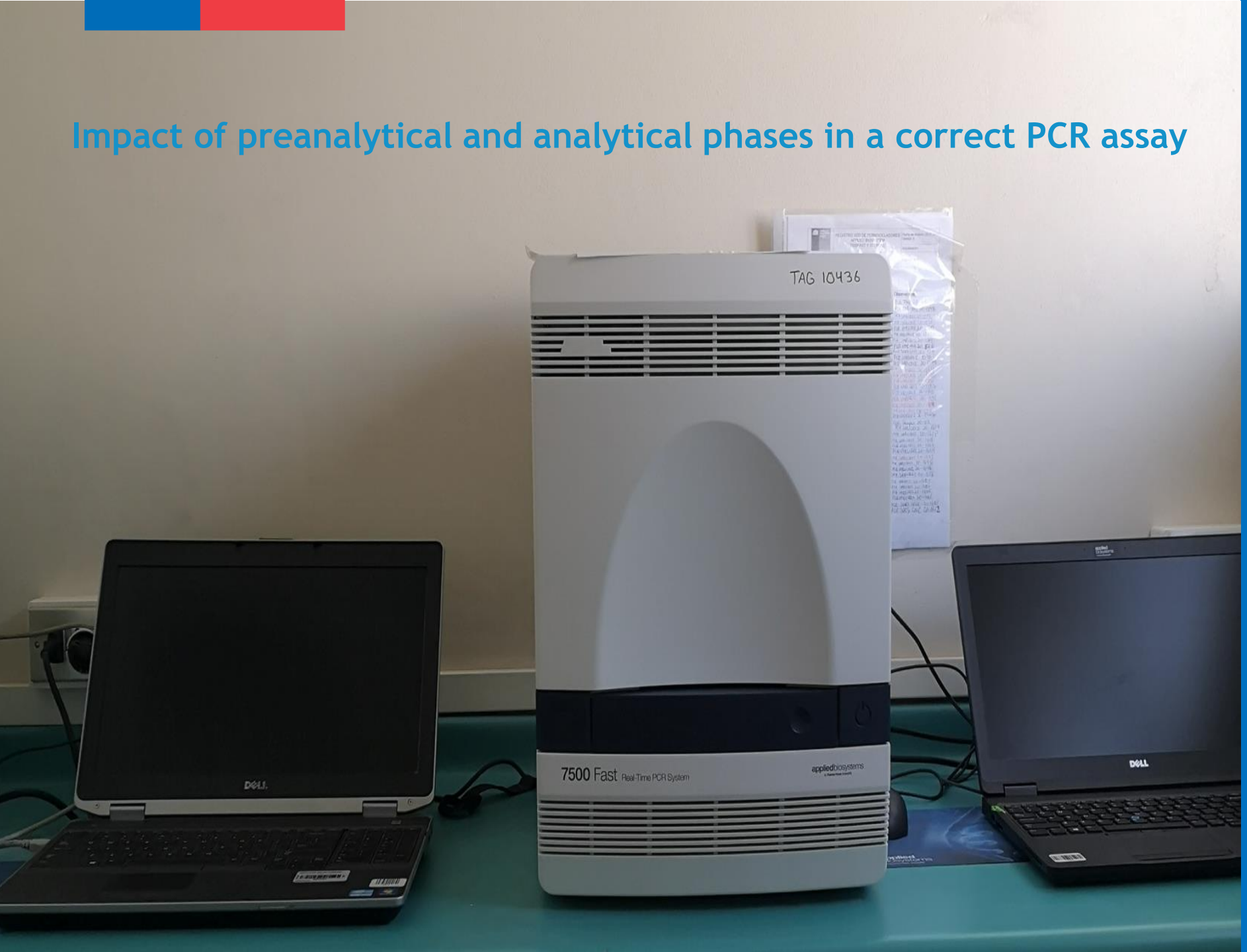
As laboratory equipment, automatic pipettes, in the same way pipette tips are consumables that must be verified in their suitability and their metrological traceability must be ensured so that volume measurements are correct and do not affect the results issued, and therefore they can affect the clinical diagnosis of a patient.

SARS-CoV-2 RT-PCR ANALYSIS & VOLUME MEASUREMENT

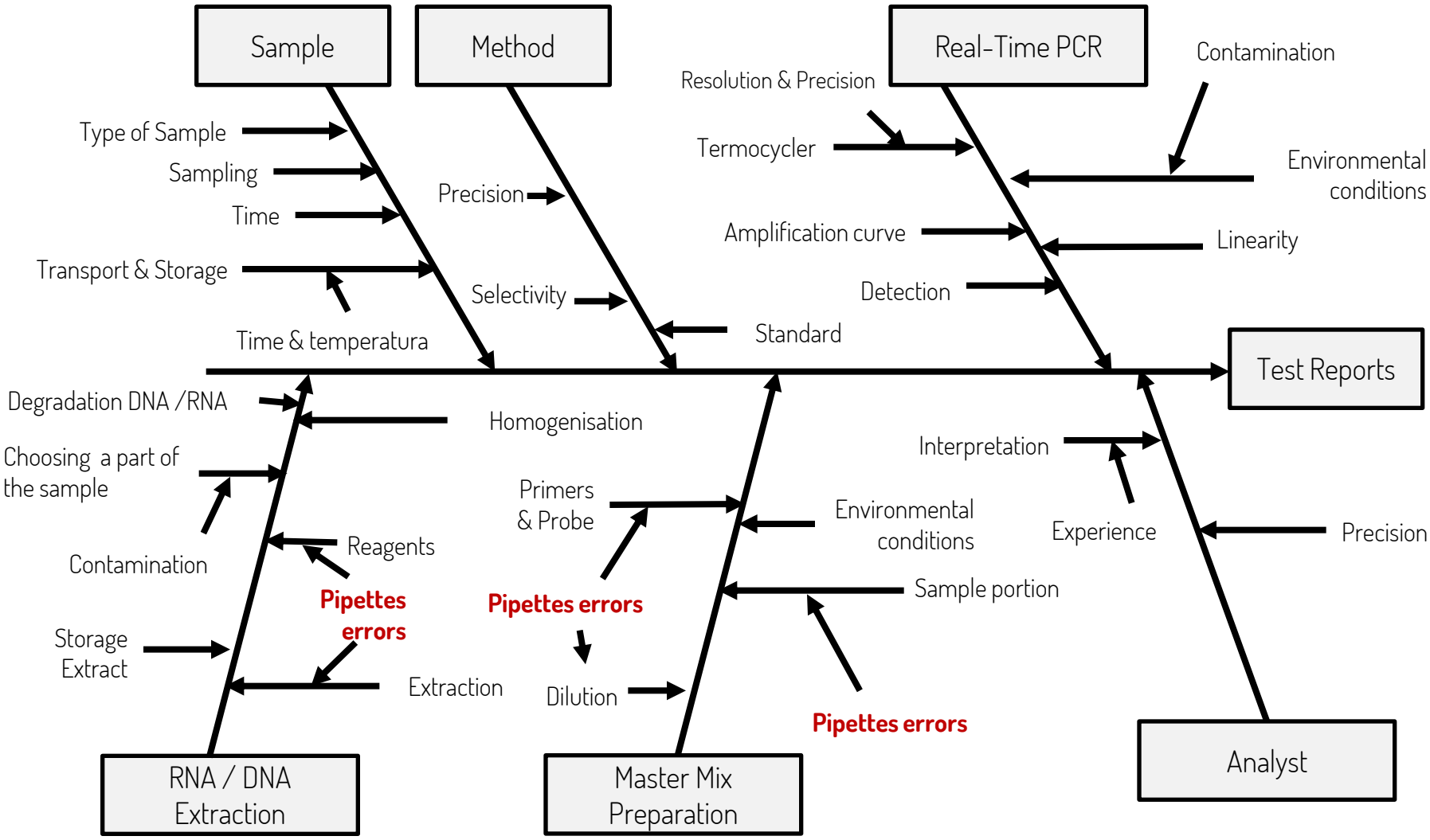
PCR analysis involves the measurement of micro volumes of samples and reagents (5 to 1000 µL) for the purpose of RNA extraction and subsequent amplification and thus reach the diagnosis.

For this purpose, automatic variable or fixed volume pipettes are used, and in the same way these can be single-channel or multi-channel. **The volume measurement capacity of each type of pipette will imply an accuracy and precision that depends on the measurement range.** Therefore, it is important to know the measurement process in which it is involved and a survey of the calibration and / or verification needs of the micropipettes used by national clinical laboratories.

Impact of preanalytical and analytical phases in a correct PCR assay



Cause - Effect Chart ⇒Uncertainty measurement in RT-PCR



Diagnostic tests for covid-19—improving accuracy and global harmonisation

May 6, 2020

As the epidemic outbreaks of novel respiratory tract infectious diseases SARS, MERS, and the ongoing pandemic of covid-19 have shown, the development of accurate diagnostic tests play an important role in outbreak management.[1,2]

Currently recommended molecular assays[4-6] detect different regions of the SARS-CoV-2 viral genome. While this can provide resilience by accounting for sequence variation between populations, it can also lead to diagnostic discrepancies associated with genomic variability or analytical performance. Often overlooked are the pre-analytical and processing steps of the recommended protocols, these include specimen sampling tools and techniques, storage and transport, extraction required prior to performing the molecular assay. Guidelines for standardisation of molecular assays developed in response to emerging infectious diseases are required. The potential impact of ignoring this is well known. When using non-standardised molecular assays for viral analysis, differences in excess of a hundred-fold are not uncommon[8] and an artificially low signal (e.g. due to poor sample processing) can manifest as a false negative result.

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Timothy D McHugh, *Professor of Medical Microbiology & Director UCL* Centre for Clinical Microbiology, *University College London, UK.*

Jacob Moran-Gilad, *Ben-Gurion University of the Negev, Israel.*

Alimuddin Zumla, *University College London, UK, and London Hospitals NHS Foundation Trust, UK.*

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The different types of methodologies for PCR, different protocols, equipment, pipettes, etc. the probability of having errors in the results increases, when there is no standardization in these aspects.

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Opinion Paper

Giuseppe Lippi*, Ana-Maria Simundic[a] and Mario Plebani[a]

Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19)

Problems may occur in COVID19 analysis assays in the pre-analytical and analytical stage due to various causes, among these are the inadequate measurement of volumes due to pipetting errors.

[a]Ana-Maria Simundic and Mario Plebani share senior authorship in this work.
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Table 1: Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19) using (real time) reverse transcription polymerase chain reaction (rRT-PCR).

Preanalytical
General
– Lack of identification/misidentification
– Inadequate procedures for specimen (e.g. swab) collection, handling, transport and storage
– Collection of inappropriate or inadequate material for quality or volume
– Presence of interfering substances
– Manual (pipetting) errors
Specific
– Sample contamination
– Testing in patients receiving antiretroviral therapy
Analytical
– Testing carried out outside of the diagnostic window
– Active viral recombination
– Use of non-adequately validated assays
– Lack of harmonization of primers and probes
– Instrument malfunctioning
– Insufficient or inadequate material
– Non-specific PCR annealing
– Misinterpretation of expression profiles

PREANALITYCAL

ANALITYCAL

Muestra de Hisopado Nasofaríngeo

Extracción del RNA Manual

Extracción del RNA Automatizado

Suspensión RNA COVID 19

Suspensión RNA COVID 19

Preparación de Reactivos

Preparación del Mix

PCR tiempo real RT-PCR COVID 19

Type Pipettes

Multi pipettes 100 to 1000 µL
Single pipettes 100 to 1000 µL
Single pipettes 10 to 100 µL

Substance:
Patient Sample
Water
Buffer
Others

Extracción

Type Pipettes

Multi pipettes 10 to 100 µL
Single pipettes 100 to 1000 µL
Single pipettes 50 to 200 µL
Single pipettes 50 to 200 µL

Substance:
Suspension
reagents

Preparación de Mix

Amplificación y visualización del producto PCR

Effects of micro-volume measurements on RT-PCR

The erroneous measurement of volumes in the measurement process could generate:

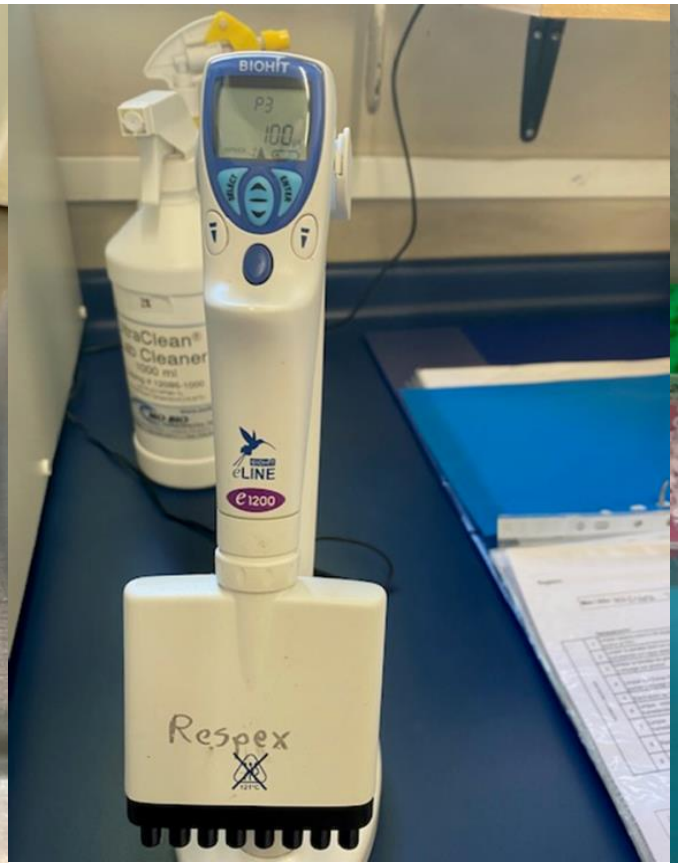
- Default errors in the aliquot taking of patient samples.
- Incorrect preparation of PCR reagents, altered concentrations.
- Effects on the measurement amplification process, inhibition of amplification, affecting the result (false negatives).
- Incorrect measurement of results due to inexperience of the analyst in the use of automatic pipettes.
- Loss of samples or reagents, due to pipette tips being unsuitable for the equipment.

Analyst Errors (pipette operator)

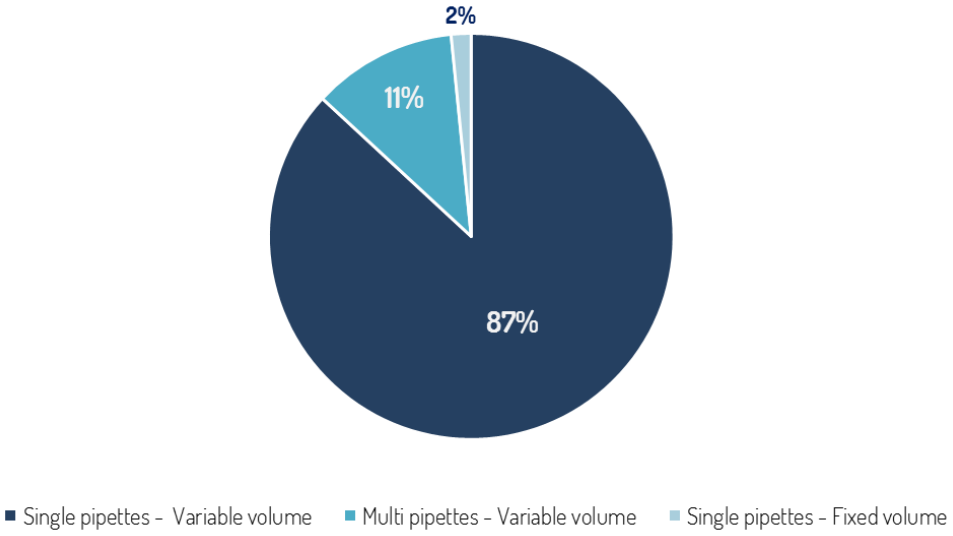
Team Errors (Non-compliant pipette)

Types of pipettes used by ISP Clinical laboratories

A survey of the types of micropipettes used by the Clinical laboratories of the National Biomedical Reference Laboratory of the ISP was carried out, and in this way it can be projected what type of needs at the national level.



Type of Automatic Pipettes used in Clinical Laboratories (ISP)- 2020

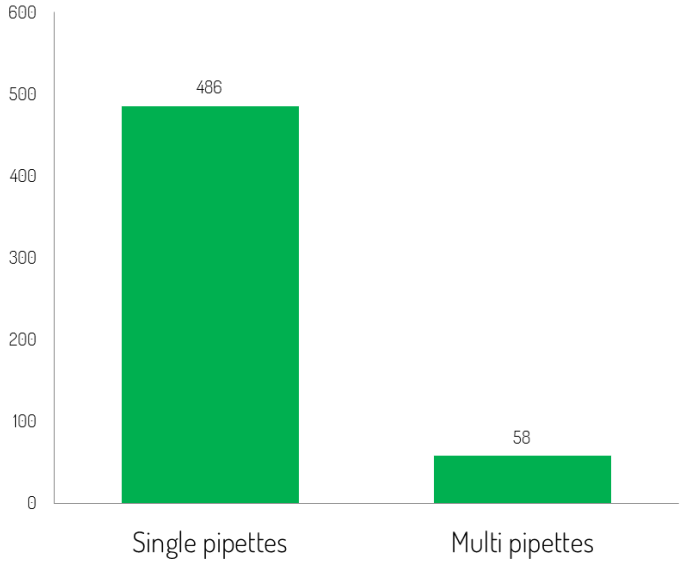


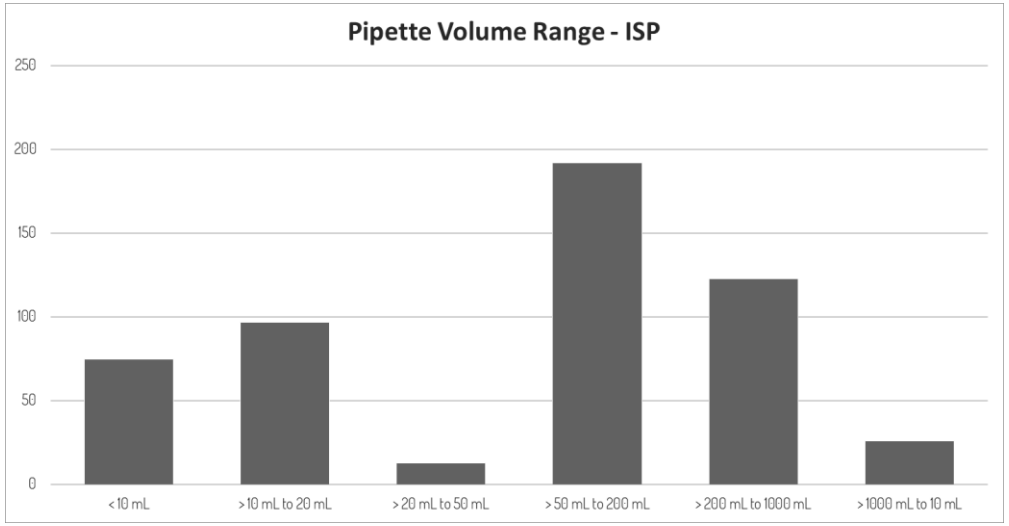
The ISP clinical laboratories in their different sections have about 500 pipettes.

Pipettes of various types are used, therefore, the calibration and/or verification requirements differ based on the measurement range.

89% single pipettes.

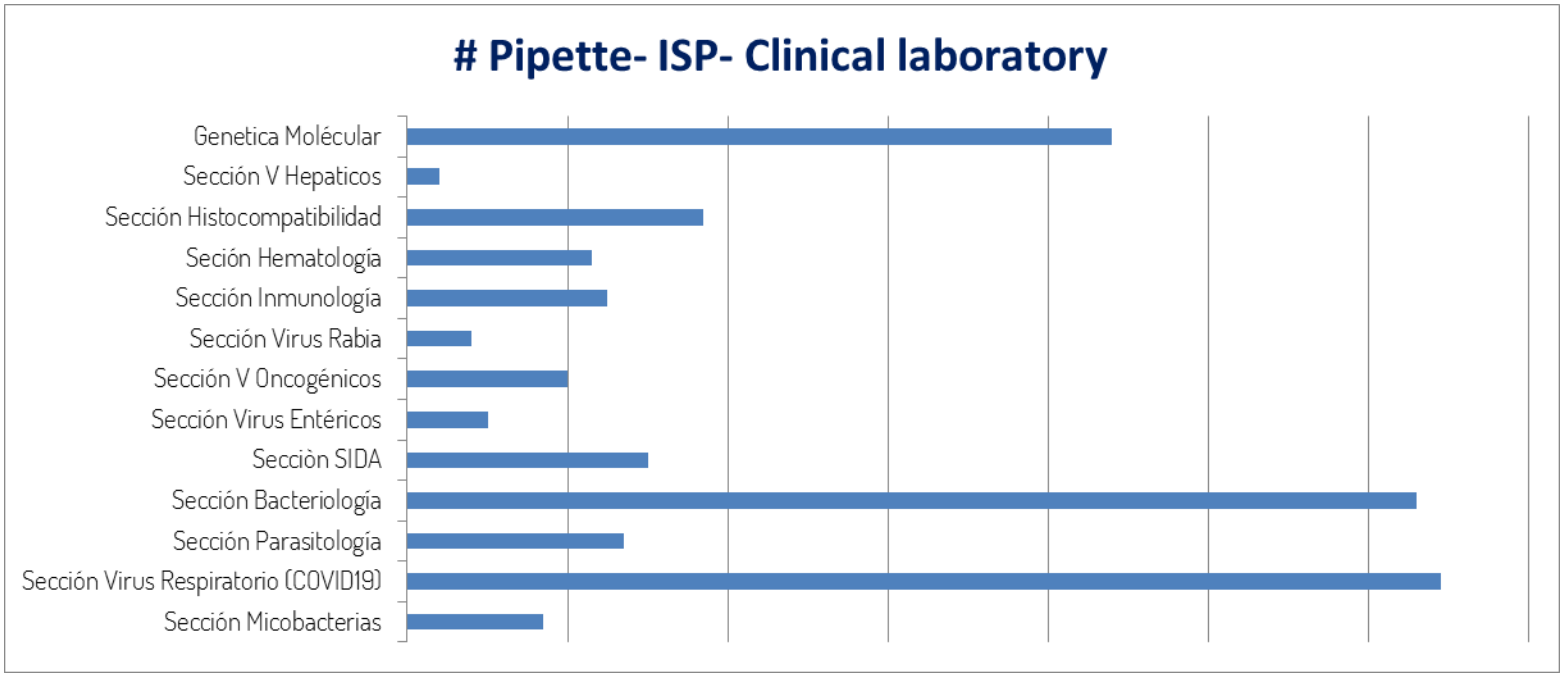
Type pipette- ISP

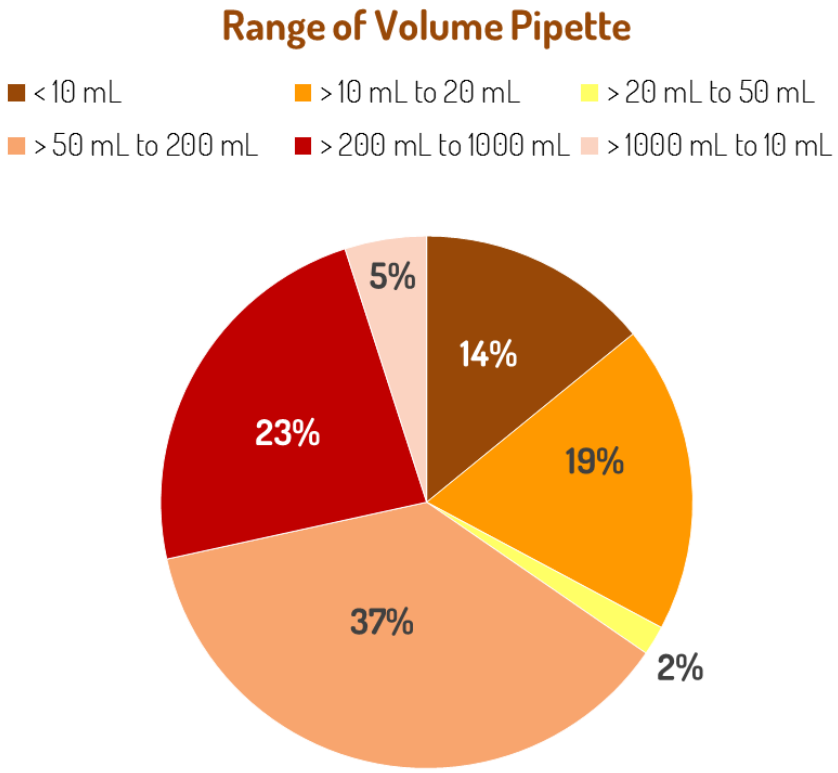




The most commonly used pipettes are those from 50 µL to 200 µL.

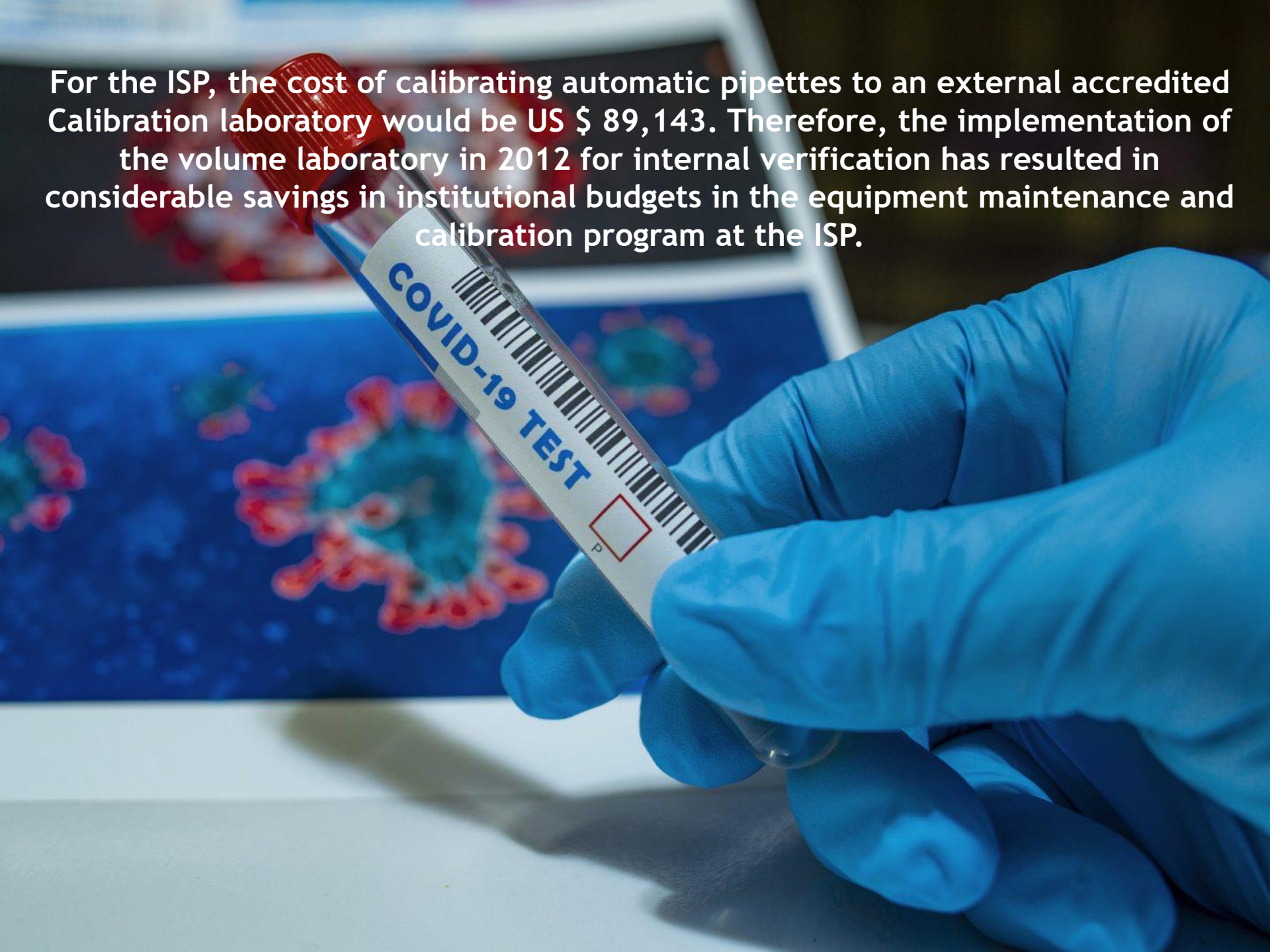
On average a clinical laboratory could have 40 automatic pipettes.





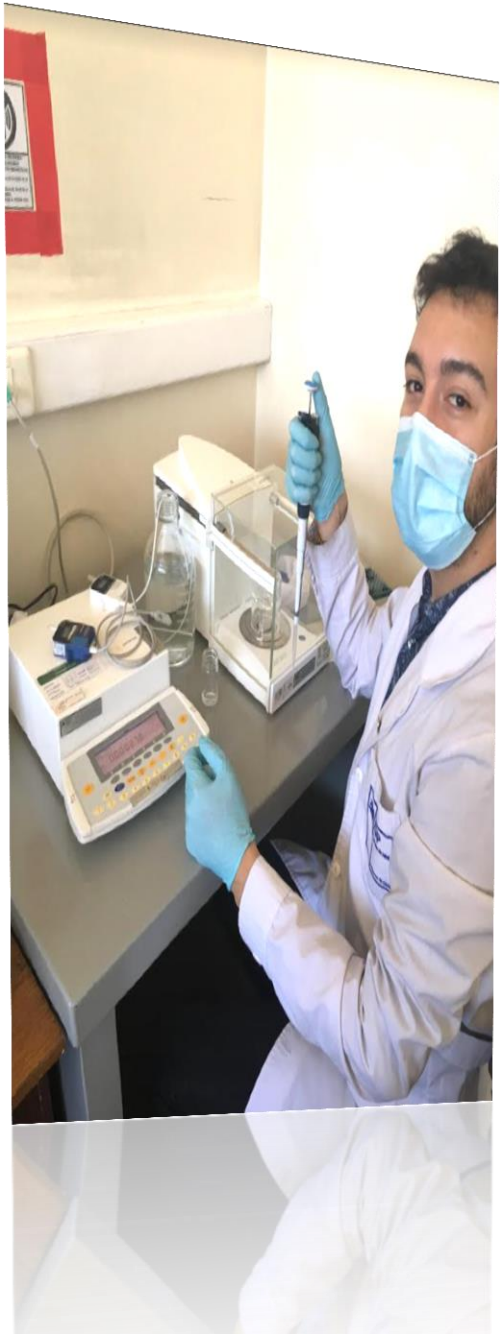
The ISP clinical laboratories have around 500 automatic pipettes. In Chile there are around 895 laboratories and blood banks, for which the projected would be around 35,800 pipettes nationwide for use in clinical assay.

- **33% of the pipettes used have a capacity <20 µL.**
- **62% are> 20 µL to 1000 µL**
- **Only 5% of pipettes are used for measurements of volumes greater than 1000 µL.**
- **Volume aliquots in assays can range from 2 uL to 10 mL**
- **25% of the total pipettes belong to the ISP Section in charge of COVID 19 analysis.**



For the ISP, the cost of calibrating automatic pipettes to an external accredited Calibration laboratory would be US $ 89,143. Therefore, the implementation of the volume laboratory in 2012 for internal verification has resulted in considerable savings in institutional budgets in the equipment maintenance and calibration program at the ISP.

Needs Detected



The main needs that were detected and / or addressed:

- → Definition by the National Biomedical Reference Laboratory of personnel in charge of the pipette verification process.
- → An annual verification frequency was established.
- → Establishment by the Physical Metrology Unit for intermediate verification of automatic pipettes.
- → Reinforcement of good laboratory practices in the use of automatic pipettes.
- → Training of personnel in Clinical Laboratories of the ISP in GLP of balances and operation of Semi-Micro Balance and in the pipette verification process by the Physical Metrology Unit of the Designated Laboratory.
- → Training of analyst personnel on metrological traceability, use and Interpretation of calibration certificates according to ISO / IEC 17025, and use of control charts.
- → Metrological traceability of balance for verification of pipettes through the Designated Mass Laboratory CESMEC- RNM Chile.
- → In process, improvement of verification and calibration capabilities of volumes <20 μL.


AirClean 600

Conclusions

- The ISP Designated Metrology Laboratory, with the support of the Mass Designated Laboratory CESMEC, developed the calibration capabilities of automatic pipettes.
- The personnel of the Physical Metrology Unit were trained in calibration of volumetric material and pipettes at LACOMET.
- The Physical Metrology Unit The ISP's Designated Metrology Laboratory had to strengthen the knowledge and skills of the ISP's clinical laboratory personnel in charge of the use of automatic pipettes, balances and the importance of metrological traceability.
- A work program was established under coordination with the Physical Metrology Unit of the Designated laboratory for the purpose of carrying out the annual verifications.
- With the support of the CESMEC Designated Mass Laboratory, the requirements of the environmental and gravimetric conditions of the Volume Room were defined for the purposes of measurements below 20 μL.

Metrology is a tool essential in the development of Scientific information reliable and comparable in health

Science at the Service of Health

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