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SARS-CoV-2 RT-PCR test circuit: Experience of Institute Pasteur de Côte d'Ivoire

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Abstract

The COVID-19 pandemic is caused by a microorganism in the Coronavirus family, SARS-CoV-2. It began in December 2019 in Wuhan, China, before spreading very rapidly to all regions of the world. In Côte d'Ivoire, the first case was detected on 11 March 2020. To combat this pandemic effectively, the government has drawn up a response plan. The *Institut Pasteur de Côte d'Ivoire* (IPCI) has been mandated to carry out RT-PCR analyses on all samples taken in the centers set up for this purpose. In carrying out this mission, the IPCI has mobilised several of its units, whose tasks include reception, decontamination, aliquoting, extraction, amplification, data entry and validation of results. These various tasks make up the sample circuit in the Institute. To curb this pandemic, the World Health Organization (WHO) has recommended early and timely identification of infected people by laboratory diagnosis using Nuclear Acid Amplification Tests (NAATs).

In this context, the IPCI has acquired a number of cutting-edge pieces of equipment, including automatic extractors, amplifiers and high-throughput sequencers. This equipment has enabled it to carry out more than one million six hundred thousand RT-PCR tests, or 98% of the tests carried out nationwide. By adhering to this schedule, we were able to meet the government's requirements, which were to deliver quality results within a short timeframe to patients, contact subjects and prospective travellers.

Keywords: COVID-19; IPCI; RT-PCR tests; Circuit; Samples

1. Introduction

Coronavirus 2019, or COVID-19, is an emerging infectious disease caused by a microorganism of the Coronavirus family, SARS-CoV-2 [1]. It began in China's 7th most populous city, Wuhan. This city witnessed the expansion of the COVID-19 epidemic, which spread throughout China and then to many countries around the world [2]. On 11 March 2020, the WHO declared COVID-19 a global pandemic [3; 4].

Faced with this health crisis, diagnoses must be appropriate and precise, and screening must be as effective as possible. All of this requires laboratory tests that are adapted and effective depending on the situation [5]. In this context, the reference tests for laboratory diagnosis are direct virus detection tests, because of their high sensitivity in the early stages of the disease.

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From the start of the pandemic, the choice quickly fell on the detection of specific viral sequences using Nucleic Acid Amplification Tests (NAATs). This technique, essentially based on Polymerase Chain Reaction (PCR) in real time after reverse transcription (RT-PCR), has been validated for diagnostic purposes and represents the Gold Standard Diagnosis for detecting any individual carrying Sars-CoV 2 [6].

In the context of the COVID-19 pandemic, the nature of the biological samples handled required maximum biosafety. Consequently, molecular diagnostics could only be carried out in specialised laboratories that met very strict safety and organisational standards. In this context, according to [7], protective measures are determined on the basis of risk assessment. High-risk samples such as nasopharyngeal swabs are analysed in level 2 Biological Safety Laboratories (LSB2), in compliance with the rules of good laboratory practice.

In Côte d'Ivoire, the first case was detected on 11 March 2020. In order to combat this pandemic effectively, the Ivorian government has drawn up a response plan, set out in [8]. The *Institut Pasteur de Côte d'Ivoire* (IPCI) has been mandated to carry out laboratory diagnosis of SARS-CoV-2 on all naso-pharyngeal samples taken at screening centers set up for this purpose.

In carrying out this mission, the IPCI has mobilised several of its specialist units, whose tasks include reception, decontamination, aliquoting, extraction, amplification, data entry and validation of results.

All these tasks are part of the different phases of sample processing in the laboratory, in particular the pre-analytical, analytical and post-analytical phases.

The aim of this paper is to highlight one aspect of the managerial approach implemented by the IPCI during this pandemic.

2. Different stages of sample processing

In Côte d'Ivoire, as in many other countries around the world, the specific diagnosis of COVID-19 is carried out by RT-PCR on nasopharyngeal samples. The handling of COVID-19 samples and the sanitary rules applicable to laboratory staff generally follow the same recommendations as those established for pathology laboratories [9;10]. For sample processing, it is recommended that personal protective equipment (PPE) such as FFP2 masks, protective visors, goggles, Tyvek-type coveralls, disposable medical gowns, gloves and gowns be worn.

2.1 Pre-analytical phase

2.1.1 Sampling

Nasopharyngeal swabs are taken in dedicated centre under the lead of the *Institut National de l'Hygiène Publique* (INHP) by authorised health personnel in an isolated area, in compliance with safety conditions (wearing of personal protective equipment) and biosafety requirements.

After sampling, the swabs are placed in tubes containing viral transport medium (VTM). These tubes are first placed in 95 kPa plastic bags with blotting paper, then placed in category B packaging (UN 3373) containing cold accumulators. These samples are sent to the IPCI as quickly as possible. The use of this triple packaging is of vital importance in effectively reducing risks during the transport of biological samples.

2.1.2 Reception, sorting and decontamination

A traceability system has been put in place to ensure that samples are properly tracked throughout the laboratory, from receipt to delivery of results.

On arrival, samples are received and recorded by the dedicated team. Before analysis, checks are carried out on the origin, number, QR code and presence of the swab in the tube, to assess the sample's conformity. After these checks, the time of delivery and the name of the carrier are recorded in an appropriate register.

Sampling tubes with no swab, no transport medium and no QR code are classified as non-compliant and removed from the batch of samples to be analysed. After this sorting, the compliant samples are decontaminated and continue the circuit.

2.1.3 Aliquoting

The decontaminated, compliant samples are given to the aliquoting team. This involves segmenting the primary sample into one or more secondary containers while ensuring traceability back to the original sample. Two essential activities are carried out by this team:

Making aliquots of 1.5 mL and matching the barcodes (codes with which the samples arrived at the IPCI) with the codes allocated to the laboratory.

These tasks enable traceability from the primary sample.

2.2 Analytical phase

This phase consists of two steps: extraction of the Ribonucleic Acid (RNA) and its amplification by RT-PCR.

2.2.1 RNA extraction

This task is carried out by teams in two departments, the Environment and Health Department and the Epidemic Virus Department. The viral RNA extraction and purification procedure comprises four main stages, namely lysis of the cells contained in the nasopharyngeal specimen, binding of the nucleic acids to the magnetic particles, washing followed by elimination of the cellular debris and elution of the nucleic acids.

Viral inactivation or lysis of viral cells

Virus inactivation consists of the lysis of SARS-Cov 2 cells by the chemical method, following the instructions of the manufacturer of the extraction kit used.

This stage is carried out in a Microbiological Safety Station (MSS) to protect the operator and the environment from the dangers of aerosols produced by handling the virus.

Extraction

Once inactivated, the samples are processed according to the rules of molecular biology. In the case of SARS-Cov 2, the genetic material is RNA. It is extracted using automated machines. The RNA extracts obtained are stored in a cooler containing cold accumulators. They are then transferred to the team responsible for amplification.

Amplification by RT-PCR

All the protocols are based on the amplification of conserved SARS-CoV-2 genes. These are mainly the N gene, which codes for the nucleocapsid proteins, the RdRp gene and the E gene, which codes for the envelope protein. The analytical sensitivity of reagents targeting the RdRP and E genes is higher [11].

2.3 Post-analytical phase

2.3.1 Data management

Two essential tasks were assigned to the data management team. Firstly, to establish the correspondence between the initial barcodes and the codes generated in the laboratory, and secondly, to electronically archive the results.

2.3.2 Publication of results

The final stage in the circuit is validation of the results, after which they are published. This publication enabled patients to receive their results on their smartphones.

3. Conclusion

The managerial approach implemented by the IPCI through well-defined logistical management of the sample circuit enabled more than one million six hundred thousand RT-PCR tests to be carried out between March 2020 and May 2022. Adherence to this circuit has enabled the IPCI to meet the government's requirement for quality results to be delivered quickly to patients, contacts and potential travellers.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare no conflict of interest.

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