

# THE SPREAD OF SARS COV-2 VIRUS IN THE POLLOG REGION DURING THE 2020 PANDEMIC

Nora ABDIU<sup>1\*</sup>, Dije DEHARI<sup>1</sup>, Muhamed SHEHABI<sup>1</sup>, Neset IZAIRI<sup>2</sup>, Shefket DEHARI<sup>1</sup>

<sup>1</sup>Departamet of Chemistry, Faculty of Natural Sciences and Mathematics, University of Tetovo, 1200 Tetovo, North Macedonia

<sup>2</sup>Departamet of Physics, Faculty of Natural Sciences and Mathematics, University of Tetovo, 1200 Tetovo, North Macedonia

\*Corresponding Author: e-mail: nora.abdiu@unite.edu.mk

---

## Abstract

SARS-CoV-2 is a single-stranded ribonucleic acid (RNA) virus with an approximately 30kb genome. For the detection of the SARS-CoV-2 virus, the detection of viral RNA through nucleic acid amplification (NAAT), such as RT-PCR (real-time polymerase chain reaction), remains a reference standard.

Samples of patients tested for SARS CoV-2 virus at the IPH Laboratory for Molecular Diagnostics "LAOR" have been used for the realization of this scientific research. This research covers Polog region patients' data in the period from July 1st through December 31st of 2020. The samples were taken from the nasopharyngeal and oropharyngeal swabs, which then were placed in the viral RNA extraction facility. This procedure was performed through the automated medical equipment: QIAcube Connect (Qiagen) and the kit of reagents: QIAamp Viral RNA Mini QIAcube Kit (Qiagen). The basis of the extraction is the binding of the viral RNA specifically to the silica membrane of the QIAamp. The extracted RNA is amplified and detected through the PCR method. For the amplification process, the QIAquant 96 5plex (Real-Time -PCR)-Qiagen medical device was utilized, as well as the following set of reagents: Primerdesign™ Ltd Coronavirus COVID-19 genesig® Real-Time PCR assay (Real-Time PCR reagent) and EURORealTime SARS-CoV-2 (Real-Time PCR reagent).

The obtained results of SARS-CoV-2 viral RNA detection show that of the total number of those tested, only 14.04% were positive. More people were tested in October 2020. Gender-wise, more men were affected, who differ in percentage from the female gender by only 2.82%. Regarding the age group, people over 50 were more affected with a percentage of 40.98%, then the age group of 31-50 years with 35.43%, then the age group of 19-54% and the less affected age group under 18 years with a percentage of 4.06%

*Keywords:* pandemics, SARS-CoV-2, Real-Time -PCR, Molecular Diagnostics, viral RNA.

---

## 1. Introduction

The year 2020 will be remembered as the start of the COVID-19 pandemic, which brought about a turning point in global health and highlighted how quickly the world can be overwhelmed by a pathogen.

Although the pandemic brought a very difficult period for the world in all aspects, it also signaled how science can react to this situation. In a few words, the pandemic has opened up great scientific opportunities and it has benefited from them, such as: the technological revolution, vaccine development, the genomic sequencing of SARS-CoV-2 and the development of testing procedures.

COVID-19 affects different people in different ways. Most infected people will develop mild to moderate illness and recover without hospitalization. People with mild symptoms who are otherwise healthy should manage their symptoms at home. On average it takes 5–6 days from when someone is infected with the virus for symptoms to show, however it can take up to 14 days.

Official names have been announced for the virus responsible for COVID-19 (previously known as "2019 novel coronavirus") and the disease it causes. The official names are:

---

<sup>1</sup> SARS-CoV-2 genomic sequencing for public health goals, World Health Organization, Interim guidance 8 January 2021

<sup>2</sup> Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health, World Health Organization, 8 January 2021

**Disease:** coronavirus disease (**COVID-19**)

**Virus:** severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**).<sup>3</sup>

## 2. What is SARS-CoV-2?

SARS-CoV-2 is a single-stranded Ribonucleic Acid (RNA) virus of the genus Betacoronavirus of the Coronaviridae family, with a genome of approximately 30 kb. The SARS CoV-2 genome encodes non-structural proteins, four structural proteins (spike [S], envelope [E], membrane [M], nucleocapsid [N]) and several accessory proteins.

Entry into the host cell, SARS-CoV-2 requires binding of the S protein to the host cell's angiotensin-converting enzyme 2 (ACE-2) receptor.

*2.1. Types of testing :* Testing is a critical tool in detecting the etiological agent, understanding virus transmission, and guiding and monitoring public health control measures and the clinical management of patients. Several different diagnostic techniques for SARS-CoV-2 are available, as follows:

- Detection of viral RNA, through manual or automated nucleic acid amplification tests (NAAT), such as real time reverse transcription polymerase chain reaction (RT-PCR);
- Detection of viral antigens through immunodiagnostic techniques, such as lateral flow assays (LFAs), commonly called rapid diagnostic tests or Ag-RDTs.
- Detection of host antibodies through serological techniques, such as LFAs, enzyme linked immunosorbent assays (ELISAs), or chemiluminescent immunoassays (CLIAs).

NAAT is the most sensitive and specific and is therefore recommended as the reference standard.<sup>4</sup>

### *2.2. What is the RT-PCR?*

Real time PCR (RT-PCR) is a nuclear-derived method for detecting the presence of specific genetic material in any pathogen, including viruses.

A sample for the SARS-CoV-2 virus is taken from the nasopharyngeal and oropharyngeal area, which is treated with chemical reagents that remove substances such as proteins and fats and extract only the RNA present in the sample. This extracted RNA is a mixture of a person's genetic material and, if present, viral RNA.

RNA is reverse-transcribed into DNA using a specific enzyme. Short DNA fragments (with fluorescent dyes) that are complementary to specific pieces of transcribed viral DNA are then added. If a virus is present in the sample, these fragments attach to targeted sections of viral DNA.<sup>5</sup>

The mixture is placed in an RT-PCR machine. The machine cycles through temperatures that heat and cool the mixture to trigger specific chemical reactions that create new, identical copies of the target sections of viral DNA. The cycle is repeated over and over to continue copying the target sections of viral DNA.

As new copies of the viral DNA sections are built, the marker labels attach to the DNA strands and then release a fluorescent dye, which is measured by the machine's computer and presented in real time on the screen. The computer tracks the amount of fluorescence in the sample after each cycle. When a certain level of fluorescence is surpassed, this confirms that the virus is present.<sup>6</sup>

---

<sup>3</sup> Recommendations for national SARS-CoV-2 testing strategies and diagnostic capacities, World Health Organization, Interim guidance 25 June 2021

<sup>4</sup> Coronavirus disease (COVID-19), Situation Report – 185, Data as received by WHO from national authorities by 10:00 CEST, 23 July 2020

<sup>5</sup> How is the COVID-19 Virus Detected using Real Time RT-PCR?, Nicole Jawerth, International Atomic Energy Agency, 27 March 2020

<sup>6</sup> Understanding COVID-19 PCR Testing, National Human Genome Research Institute (genome.gov)

2.3. *Result interpretation of the PCR test for COVID-19:* A positive result occurs when the SARS-COV-2 fluorescent primers bind to the DNA in the sample and the sequence is amplified, so it is unlikely that a sample will be positive if viral RNA is not present. If it does, this is a false positive.

A negative result occurs when the SARS-CoV-2 primers do not match the genetic material in the sample and there is no amplification. A false negative result occurs when a person is infected, but there is not enough viral genetic material in the sample for the PCR test to detect.

### 3. The Experimental Part

3.1. *Materials and Methods:* The patients tested for SARS CoV-2 at PHI Laboratory for Molecular Diagnostics LAOR in Tetovo have been taken for this scientific study work. The area under study was the part of Polog Region from July 1st 2020 until December 31st 2020.

The following devices have been utilized for this process:

- QIAcube Connect (automatic viral RNA extraction device)<sup>7</sup> and
- QIAquant 96 5plex (Real-Time -PCR)<sup>89</sup>,

Whereas, the reagents that have been utilized for this process include:

- QIAamp Viral RNA Mini QIAcube Kit (240) (for viral RNA extraction)<sup>10</sup>,
- Primerdesign™ Ltd Coronavirus COVID-19 genesig® Real-Time PCR assay (Real-Time PCR reagent)<sup>11</sup> and
- EURORealTime SARS-CoV-2 (Real-Time PCR reagent)<sup>12</sup>.

3.2. *Stages of analysis:* The experimental part, from swabbing to reading the results (detection or non-detection of SARS-CoV-2 genetic sequences) includes the following stages:

- Sampling – nasopharyngeal or oropharyngeal swab for RT-PCR molecular detection of COVID-19. It is necessary to place the sample in a viral medium immediately.<sup>13</sup>
- The molecular procedure for viral RNA extraction, a procedure which is carried out in a special chamber – the entire process of manipulating the samples is carried out in Biosafety Cabinet Class II with heap filters by personnel that has gone through training to work and handle highly infectious materials.
- After the extraction of the viral RNA, the second stage is the preparation of the specific Master Mix with appropriate primers and controls and the passage of the extracted RNA to the second special space of the molecular laboratory, under strictly controlled conditions and defined procedures of work in it. The person in charge of this procedure has gone through internal trainings for personal protection while working with or handling highly infectious materials.<sup>14</sup>
- The third stage is the beginning of the application process of the RT PCR instrument, where the instrument is initially calibrated to detect a suitable spectrum for the specific virus detection. Programming of samples that will be subjected to amplification, virus-specific target detection is done manually.

---

<sup>7</sup> QIAcube Connect, [www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/instruments-equipment/qiacube-connect](http://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/instruments-equipment/qiacube-connect)

<sup>8</sup> QIAquant™ 96, Software User Manual, July 2021

<sup>9</sup> QIAquant™ 96, User Manual, July 2022

<sup>10</sup> QIAamp® Viral RNA, Mini Handbook, July 2020

<sup>11</sup> Primerdesign™ Ltd, Coronavirus COVID-19, genesig® Real-Time PCR assay, Issue 4.01, 24th July 2020

<sup>12</sup> EURORealTime SARS-CoV-2, MP\_2606\_A\_US\_D03.doc, x Version: 2022-04-14

<sup>13</sup> Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing, October 25, 2021

<sup>14</sup> Coronavirus disease (COVID-19) outbreak: rights, roles and responsibilities of health workers, including key considerations for occupational safety and health. Interim guidance, 18 March 2020. Geneva: World Health Organization

- The fourth stage is the result interpretation. Simultaneous controls should be issued for each sample: negative controls, positive controls and controls for monitoring RNA extraction, the reverse transcriptase process.
  - Results are read and interpreted according to the instructions provided by the reagents manufacturer. They are accepted as valid if the preanalytical phase is fully respected by taking a quality nasopharyngeal and oropharyngeal swab, an accurate and precise analytical phase, and valid results for each control sample.

#### 4.The Obtained Results

*4.1. The results obtained:* For research purposes, 8607 samples were taken from the period 01.07.2020-31.12.2020 of the Pollog region, for detection or non-detection of SARS-CoV-2 viral RNA. The samples were taken and processed at the PHI Laboratory for Molecular Diagnostics “LAOR” in Tetovo.

The data are grouped per the month of testing, gender and age groups. The total number of those tested and those who were positive, the total number of male and female patients were counted and selections were made depending on age groups.

The age groups are divided as follows:

- under 18 years old,
- 19-30 years old,
- 31-50 years old and
- over 50 years old.

In July (July 01, 2020-July 31, 2020), 815 patients were tested, of which 99 were positive. From the total number of those tested, 12.15% are positive, 35.35% are women and 64.65% are men. Among the age groups most affected by the SARS-CoV-2 virus is those over 50 years old (with 40.40%), while the age group between 18 years old (with 4.04%) is the least affected.

In August (August 01, 2020-August 3, 2020), 1772 patients were tested, of which 107 were positive. Of the total number of those tested, 6.04% are positive, 40.19% are women and 59.81% are men. Among the age groups most affected by the SARS-CoV-2 virus is that between 31-50 years old (with 35.51%), while the least affected is the age group between 18 years old (with 8.41%). Compared to July, August showed an increase in the total number of people tested, while a decrease in the percentage of positive cases.

In September (September 01, 2020- September 30, 2020) 1282 patients were tested, of which 65 were positive. Of the total number of those tested, 5.07% are positive, 52.31% are women and 47.69% are men. Among the age groups most affected by the SARS-CoV-2 virus is that over 50 years old (with 47.69%), while the least affected is the age group between 18 years old (with 10.77%). Compared to August, in September there was a decrease in the total number of people tested and a decrease in the percentage of positive cases. It is noted that compared to the two months (July and August), in September we have an increase in the percentage of women with a positive result.

In October (October 01, 2020- October 31, 2020), 1847 patients were tested, of which 273 were positive. Of the total number of those tested, 14.78% are positive, 52.01% are women and 47.99% are men. Among the age groups most affected by the SARS-CoV-2 virus is that between 31-50 years old (with 40.29%), while the least affected is the age group between 18 years old (with 5.13%). Compared to September, in October there was an increase in the total number of people tested and a threefold increase in the percentage of positive cases. It is noted that compared to the three months (July, August and September), in October we have an increase in the total number of tested and positive cases.

In November (November 01, 2020- November 30, 2020) 1705 patients were tested, of which 498 were positive. Of the total number of those tested, 29.21% are positive, 51.81% are women and 48.19% are men. Among the age groups most affected by the SARS-CoV-2 virus is that over 50 years old (with 46.39%), while

the least affected is the age group between 18 years old (with 1.61%). Compared to October, in November there was a slight decrease in the total number of people tested and a double increase in the percentage of positive cases. It is noted that compared to the four months (July, August, September and October), in November we have an increase in the number of positive cases.

In December (December 01, 2020- December 31, 2020), 1186 patients were tested, of which 166 were positive. Of the total number of those tested, 14.00% are positive, 45.18% are women and 54.82% are men. Among the age groups most affected by the SARS-CoV-2 virus are those over 50 years old (with 41.57%), while the least affected is the age group between 18 years old (with 4.22%). In comparison to November, in December there was a decrease in the total number of people tested and a double decrease in the percentage of positive cases, there was also an increase in the percentage of male positive cases

**Table 1.** Patients' results -Total 2020

Month	Total	Positive Cases	Women	Men	Under 18 y/0	19 – 30 y/o	31-50 y/o	Over 50 y/o
July 2020	815	99	35	64	4	18	37	40
August 2020	1772	107	43	64	9	28	38	32
September 2020	1282	65	34	31	7	12	15	31
October 2020	1847	273	142	131	14	57	110	92
November 2020	1705	498	258	240	8	94	165	231
December2020	1186	166	75	91	7	27	63	69
Total	8607	1208	587	621	49	236	428	495
%	100.00%	14.04%	48.59%	51.41%	4.06%	19.54%	35.43%	40.98%

## 5. Conclusion

From the total results obtained from the period 01.07.2020-31.12.2020, it can be noted that of the total number of those tested; only 14.04% were positive, while 85.96% were negative. October 2020 is the month with more tested people.

Gender-wise, more men were affected, who differ in percentage from the female gender by only 2.82%, so we can conclude that infection with the SARS-CoV-2 virus does not depend on gender.

Age-wise, people over 50 were most affected with a percentage of 40.98%, then the age group of 31-50 years with 35.43%, then the age group of 19-30 years with 19.54%, and the age group under 18 years of age was least affected with a percentage of 4.06%, we noticed that people over 31 years old were more infected and we can conclude that we have these results of positive people because these age groups are more active in terms of traveling and contacts with other people.

## References

- [1]. Coronavirus disease (COVID-19) outbreak: rights, roles and responsibilities of health workers, including key considerations for occupational safety and health. Interim guidance, 18 March 2020. Geneva: World Health Organization
- [2]. Coronavirus disease (COVID-19), Situation Report – 185, Data as received by WHO from national authorities by 10:00 CEST, 23 July 2020
- [3]. EURORealTime SARS-CoV-2, MP\_2606\_A\_US\_D03.doc, x Version: 2022-04-14
- [4]. Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health, World Health Organization, 8 January 2021
- [5]. How is the COVID-19 Virus Detected using Real Time RT-PCR?, Nicole Jawerth, International Atomic Energy Agency, 27 march 2020
- [6]. Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing, october 25,2021
- [7]. Primerdesign™ Ltd, Coronavirus COVID-19, genesig® Real-Time PCR assay, Issue 4.01, 24th July 2020
- [8]. QIAamp® Viral RNA, Mini Handbook, July 2020
- [9]. QIAcube Connect, [www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/instruments-equipment/qiacube-connect](http://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/instruments-equipment/qiacube-connect)
- [10]. QIAquant™ 96, Software User Manual, July 2021
- [11]. QIAquant™ 96, User Manual, July 2022
- [12]. Recommendations for national SARS-CoV-2 testing strategies and diagnostic capacities, World Health Organization, Interim guidance 25 June 2021
- [13]. SARS-CoV-2 genomic sequencing for public health goals, World Health Organization, Interim guidance 8 January 2021
- [14]. Understanding COVID-19 PCR Testing, National Human Genome Research Institute (genome.gov)