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The launch of a COVID-19 diagnostic laboratory in an oncology hospital – a review of guidelines and the laboratory team's own experiences

Dagmara Michałowska[1], Aleksandra Zagrabska[1], Ewelina Czykałko[1], Natalia Skowrońska[1], Dorota Blomka[1], Krzysztof Szufnarowski[1], Ireneusz Pawlak[1], Adam Maciejczyk[1, 2], Izabela Łaczmańska[1, 3]

[1]*Wroclaw Comprehensive Cancer Centre, Wroclaw, Poland*
[2]*Department of Oncology, Wroclaw Medical University, Wroclaw, Poland*
[3]*Department of Genetics, Wroclaw Medical University, Wroclaw, Poland*

The first case of the coronavirus SARS-CoV-2 infection was confirmed in December 2019 in Wuhan, China, from whence the virus spread across the world within several weeks. Due to the alarming level of infections, the World Health Organisation (WHO) announced a SARS-CoV-2 pandemic on 12 March. This dynamic and unprecedented epidemiological situation created an urgent need to carry out SARS-CoV-2 tests in individuals meeting the criteria defined for COVID-19 suspect cases. According to the current WHO recommendations, active SARS-CoV-2 infection diagnostics is based on molecular method using a real-time reverse transcription – polymerase chain reaction (real-time RT-PCR). Highly specific and sensitive, this method makes it possible to detect even a small amount of RNA particles of the virus in the tested sample. Undoubtedly, the launch of new COVID laboratories and the implementation of adequate procedures increases the effectiveness of activities aimed at directly combatting the SARS-CoV-2 pandemic. The population of oncological patients is particularly exposed to the risk of complications and death resulting from the SARS-CoV-2 infection; therefore it is essential to ensure them the possibility of quick testing for COVID-19. This article presents the authors' own experiences as well as technical and formal issues related to the launching of a SARS-CoV-2 laboratory.

Key words: COVID-19, SARS-CoV-2, diagnostics, real-time RT-PCR

The SARS-CoV-2 pandemic, whose first case was confirmed in Poland in March 2020, forced many medical laboratories to address the need to launch departments focused on SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection diagnostics. According to the World Health Organisation recommendations, molecular tests using a real-time reverse transcription polymerase chain reaction (RT-PCR) which detect the virus genetic material in a sample collected from a patient are performed [1, 2].

The material for SARS-CoV-2 tests includes samples collected from the upper respiratory tract (nasopharyngeal swabs or tracheal and mucosal swabs) and the lower respiratory tract (trans-tracheal aspirates, broncho-alveolar lavage or non-induced sputum) [3–6].

Molecular tests are essential to detect an infection with SARS-CoV-2. At present, the number of confirmed cases in Poland exceeds 93 thousand (as of 1 October 2020), which is the result of work of over 197 laboratories. This means that in a short time many laboratories had to modify or expand the profile of their activity and adjust rooms, equipment and procedures to SARS-CoV-2 molecular diagnostics using the real-time RT-PCR method. This paper presents the experiences

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of the team involved in the launch of the COVID laboratory in Wroclaw Comprehensive Cancer Centre, Poland, which routinely used real-time PCR, PCR, Sanger sequencing and next generation sequencing methods (NGS) to detect somatic and germline mutations in oncological patients prior to the pandemic.

Cancer has been the second most common death cause in Poland. They are responsible for the death of almost 100 thousand patients per year and about 300 per day. According to the recommendations of the Polish Oncological Society (Polskie Towarzystwo Onkologiczne – PTO) and the Polish Society of Clinical Oncology (Polskie Towarzystwo Onkologii Klinicznej – PTOK), all healthcare units providing oncological treatment should implement stringent safety procedures and continue to provide healthcare services to patients during the SARS-CoV-2 pandemic [7, 8]. It is generally known that elderly persons and individuals with reduced immunity are at the highest risk of the severe form of COVID-19 and death [9]. On the basis of the Polish National Cancer Registry data, there are about one million cancer patients in Poland at present and over 60% of them are over 65 years of age, which means that the risk of complications as a result of the SARS-CoV-2 infection is severe for this population [10]. Pursuant to the current recommendations of the Chief Sanitary Inspectorate (Główny Inspektor Sanitarny – GIS) and the Health Ministry, each patient suspected of having COVID-19 should undergo tests for SARS-CoV-2 before his or her admission to an oncology centre [9]. Currently, in order to continue to provide treatment to patients at oncology centres, it is essential to ensure stringent protection systems for patients and personnel, among others by launching laboratories offering SARS-CoV-2 molecular diagnostics.

Sample collection and qualification

SARS-CoV-2 test samples are collected in a separate room which is located in an epidemic airlock within the hospital. The swab collection point is divided into 3 zones:

- the patient zone (collecting swabs),
- the working zone (sample description and packing),
- the staff changing zone (including a place where the staff members may change their clothing, a storeroom with personal protection equipment (PPE), additional materials necessary for swabs, waste disposal, etc.).

All items in the storeroom are divided into smaller packages and packed into airtight containers, which facilitates disinfection. At the beginning of the pandemic, swabs were collected in two shifts: the morning shift (8–10 AM) and the afternoon shift (1–3 PM). The morning shift was designed for the patients of the Wroclaw Comprehensive Cancer Centre in Wroclaw and the afternoon shift was for the staff working at the centre. With the stable epidemic situation at present, swabs are only collected in the morning. The staff and patients of the hospital are separated, which is also the case for symptomatic individuals. The last persons in the queue are those qualified for next test in order to confirm the virus eradication. The time between swabs (about 5 minutes) is used for surface disinfection as well as sample description and packing. Following the guidelines of the Chief Sanitary Inspector, samples are packed into three packages and stored in a fridge (located at the collection point) [5]. Swabs may also be collected at any department where employees have received the relevant training. The work schedule for the collection point staff is prepared one month ahead. Occasionally, a staff member may be sent using a hospital's means of transport to a person who is not able to come to the collection point. Each person collecting samples is equipped with PPE pursuant to the WHO guidelines (which applies both to the collection point, the ward and swabs collected outside the hospital area). The qualification of individuals for swabs is coordinated by the Hospital Infection Control Department, which verifies indications for swabs and their timing. The list of patients for admission is prepared after their qualification at outpatient clinics and the confirmation of the patient's introductory negative epidemiological history (obtained over the phone) by the secretariat of each ward.

Laboratory rooms and equipment

A COVID laboratory requires isolated rooms of biosafety level 2 (BSL – 2). Ideally, it should be located in a separate building or part of the building, but because of the sudden outbreak of the pandemic and the need to launch such laboratories quickly, this was impossible in most cases. The COVID lab should have at least 2 rooms: one for the isolation of nucleic acids, divided by an airlock, and the other one for real-time RT-PCR. Sample unpacking and virus RNA isolation should be carried out in a laminar flow cabinet of minimum biosafety level 2. Lab employees must be wearing safety clothing in the laboratory described here it includes Tyvek 500 Labo Cat. III uniforms, FFP2 or FFP3 masks and face shields, talc-free gloves, caps and shoe covers).

Each entry into and leaving of COVID laboratory rooms must be in strict compliance with very detailed safety rules. This is why SARS-CoV-2 RNA isolation is performed by one team successively for all swabs registered in a given diagnostic cycle. In this way, it is possible to reduce the need for frequent changes of protective clothing and moving between the zones separated for the purpose of diagnostics. In the early period of the pandemic, i.e. from March to May 2020, the laboratory team was divided into two smaller teams that performed tests every second day, which increased the staff's safety as these groups had no personal contact with each other. After restrictions were relaxed in June 2020, the lab went back to its standard operation. Apart from the BSL-2 laminar flow cabinet (BIO130 Alpina model), the COVID lab is equipped with the Maxwell RSC48 (Promega) device for automatic isolation of genetic material from 48 samples at the same time. Alternatively, the

genetic material can be isolated manually, but using automation, it was possible to reduce the time needed to obtain results and achieve high quality RNA. The Maxwell Blood DNA kit (Promega) and the Maxwell RSC Viral RNA kit (Promega), designed for the lab equipment, are used interchangeably to isolate RNA, depending on their availability on the market. Both have been validated by the producer as kits for the isolation of the SARS-CoV-2 genetic material and ensure the high efficiency and high quality of RNA.

According to the latest report on the results of an external quality assessment of molecular tests for SARS-CoV-2 prepared by two international organisations, the European Molecular Genetics Quality Network (EMQN) and Quality Control for Molecular Diagnostic (QCMD), the proportion of correct results obtained with the application of the Promega Maxwell RSC Viral kit is 95.5% (n = 176, p = 0.644) [11]. RNA isolation is performed according to the producer's protocol with one modification: each swab moistened with physiological saline, which is placed in a separate dry tube after material collection, is transferred to a sterile Eppendorf tube and broken off from the stick by holding the lid. Next, 300 µL of phosphate-buffered saline (PBS) is added to the tube. This step is omitted for samples collected to physiological saline. After brief mixing in a vortex, 300 µL of a buffer for lysis and 30 µL of proteinase is added. The sample is mixed in a vortex for 15 seconds and incubated for 15 minutes in the temperature of 56°C. The subsequent steps are carried out following the producer's manual. The laboratory described here decided to apply this method of collection and isolation (as compared with the isolation from physiological saline in which entire swabs are usually immersed) because it considerably facilitated the transfer of the biological material from the long tube in which the swab was placed to the Eppendorf tube and reduced the risk for the transfer of the solution potentially containing the virus onto gloves or working surfaces. According to the recommendations of the Health Minister of 21 April 2020, it is permitted to use substitutes of the equipment and/or reagents in the test method verification process without the need to carry out a full method validation if the substitute, according to the laboratory, enables the correct test performance [12]. The concentration and quality of the isolate obtained is evaluated using a NanoPhotometer N60 (Implen). Samples with low concentration, the concentration of 10 ng/µl or below or samples of poor quality are reported for another swab collection. This is especially important when there is no internal control of the housekeeping gene in the diagnostic tests used. Synthetic bacteriophage RNA added to reagents as an internal control does not make it possible to check whether the required RNA level has been achieved in the tested sample after isolation.

Apart from the basic research equipment and standard small devices (microcentrifuges, pipettes, stands, etc.) used in molecular laboratories, which must be part of the equipment in both rooms (the equipment cannot be transferred between rooms), UV flow lamps and direct UV lamps are useful for air and surface sterilisation. The advantage of UV flow lamps is that they can be turned on during the diagnosticians' work.

Swabs

The selection of swabs for sample collection from the nasopharynx in the second quarter of this year was limited due to great demand across the world. Sterile swabs must be made of artificial materials (polyester or viscose). After the selection of the type of swabs, each laboratory should check the quality of the samples collected and adjust the manner of collection to its own procedures. Due to the fact that the laboratory described here was in operation as early as in March 2020, the method of nasopharynx sample collection from healthy individuals was tested at the beginning. The total RNA from the swab, including human RNA, is isolated, so the evaluation of its concentration in the isolate made it possible to determine whether the swabs (Equimed) used were adequate. A smear was collected on a dry swab moistened just before collection with a few drops of physiological saline. Pouring 2 ml of physiological saline solution to the probe with a swab resulted in the reduced efficiency of nucleic acid isolation and impeded its first step, i.e. the separation of the swab from the stick. There were also difficulties with the transfer of the solution from the long tube containing the swab to the Eppendorf tube. The quality of the sample collected is also important. The swab should not contain blood or other contaminants (as they may contain inhibitors of the PCR reaction). Swabs were transported following the WHO guidelines and the rules specified in the document published on the website of the National Chamber of Laboratory Diagnosticians (https://kidl.org.pl/get-file/2671). Because of the limited selection of tests available on the market in the early period of the pandemic, the laboratory described here used the two-gene test Vitassay qPCR SARS CoV-2 (Vitassay) CE-IVD (genes of SARS-CoV-2: *ORF1ab* [FAM signal] and *N* gene [ROX signal] as well as an RNA internal control [HEX signal]). But this did not solve the need for the quality control of the isolated genetic material (the same results were achieved for samples without nucleic acids and for the so-called zero controls). At present, because of better parameters, the three-target test GeneFirst-Novel Coronavirus (COVID-19) Nucleic Acid Test Kit, CE-IVD (GeneFirst), is used (genes of SARS-CoV-2: *ORF1ab* [FAM signal] and *N* gene [ROX signal] as well as the human gene: *GAPDH* [CY5 signal]). The reaction was performed with the application of the CFX96™ Real-Time PCR Detection System (Bio-Rad). The detection limit for the test is 10–100 copies of the virus RNA per one reaction. The reaction is performed according to the producer's protocol for the tested samples as well as a positive control (containing synthesised sequences of the nucleic acid to detect genes ORF1ab and N of the SARS-CoV-2 virus, as well as human *GAPDH*) and a negative control (non-template control – NTC). An undeniable advantage of this kit is the detection of the GAPDH human gene, which is

the evidence for the RNA presence in the tested sample and significantly reduces the risk of a false negative result. Moreover, as has already been mentioned, the nucleic acid concentration is determined for each sample before the reaction. At the same time, along with positive and negative controls added to the kit, there is an isolation control for each series of samples (zero control), an isolation from a clean swab moistened only with sterile physiological saline. In this way, it is possible to evaluate the purity of isolation – a positive result confirms contamination and the need to repeat the entire series of tests. The quality of the isolated material depends largely on the manner of swab collection. Because the virus RNA and the patient's RNA are isolated together, there is no certainty that the sample contains SARS-CoV-2 nucleic acid despite the evaluation of the RNA concentration. This might be the reason why false negative results are obtained.

The analysis of the data obtained from real-time RT-PCR is carried out using the Bio-Rad CFX Maestro software (Bio-Rad) following the producer's manual. According to - the manual, a sample is positive when fluorescence curves for both tested viral genes have the correct shape and cross the threshold. The presence of SARS-CoV-2 is confirmed in the sample when the signal is amplified with Ct ≤ 39 in FAM and ROX channels. A sample is negative when the signal is amplified with Ct > 39.0 or without Ct in FAM and ROX channels. If one of the two tested genes produces a positive result in a FAM or ROX channel, the sample may be positive and the patient needs to be tested again. It is crucial to follow the test producer's guidelines, which enables a reduction of the risk of false positive results. Samples with a positive signal but below the threshold for which an infection onset (low viremia) may be suspected are always reported for another test in the laboratory described here. In – more than half of such cases (8/14, 57%) analysed in March–April 2020, an infection was confirmed after a few days (positive result).

It should be emphasised that the guidelines of the National Institute of Public Health – National Institute of Hygiene (Narodowy Instytut Zdrowia – Państwowy Zakład Higieny NIZP-PZH) indicate that a negative test result is not tantamount to the absence of an infection and each test result should be interpreted with reference to clinical data.

Laboratory personnel

The laboratory employs diagnosticians with extensive experience in molecular biology and two members of its staff have previously worked on molecular diagnostics of viruses. The experience of these two staff members was employed when the rooms and the layout of the equipment in the rooms for SARS-CoV-2 diagnostics were prepared and the laboratory's own decontamination procedures based on WHO recommendations were developed. Because of the need to report results to various institutions, numerous administrative employees are also involved in the work of the COVID laboratory.

Laboratory decontamination

Because of large numbers of SARS-CoV-2 samples at one time and place, the virus genome size of about 30 kb and high viremia of some patients, there is a high risk of sample contamination and false positive results regardless of the application of all possible safety measures. Each laboratory should develop and implement procedures reducing such a risk, i.e. detailed rules for the work within the BSL-2 laminar flow cabinet, handling positive control samples, handling samples from patients and decontamination of all equipment and surfaces on a regular basis.

Apart from thorough disinfection every day, it is necessary to carry out a systematic general decontamination of rooms, including surfaces and the entire equipment, on a set date. The frequency of decontamination should increase with the number of samples handled. Apart from 70% ethanol, the WHO guidelines recommend the following substances to be used for this purpose: 0.1% sodium hypochlorite (the so-called ace or bleach), hydrogen peroxide, quaternary ammonium compounds and phenolic compounds (following the producer's recommendations). Good results can also be achieved when solutions for the disintegration of nucleic acids (e.g. PDS-250 Biosan) are applied directly on surfaces in the laminar flow cabinet and on small equipment on a regular basis.

External quality control

A laboratory that performs tests for SARS-CoV-2 must be registered with the Health Ministry and undergo an external quality test offered by the NIZP-PZH in Warsaw (which is free of charge). The test involves submitting 15 of the lab's own samples (swabs or the liquid in which swabs were placed) together with the required documentation and information about the method applied. At present, international quality control programmes are also available for the purpose of SARS-CoV-2 diagnostics. Participation in such a programme significantly increases the credibility of results obtained in a laboratory. Such international organisations as EMQN and QCMD have introduced a pilot programme for the external control of the quality of diagnostic tests for SARS-CoV-2. Study results, which were published in a paper by Matheeussen et. al., present a review of the assessment carried out in 365 laboratories from 36 countries [11]. The laboratory described here has implemented a quality control system and keeps a record of pre-analytical errors. Each deviation is reported to the contracting unit and the Epidemiology Department of the Wroclaw Comprehensive Cancer Centre. If a pre-analytical or laboratory error is confirmed or results are ambiguous, the need to collect another swab is reported.

Reporting of results

An important part of the COVID laboratory's work is to report the results. Below, there is a list of web portals and institutions that require everyday reports.

- Health Ministry – reports through the portal https://wsse.mz.gov.pl (WSSE once daily (tests) at 8:00 AM and WSSE twice daily (queues) at 8:00 AM and 8:00 PM) including the number of tests available at the laboratory, the number of tests ordered individually, the number of tests performed on patients in the past 24 hours, the number of positive results in new patients in the past 24 hours, the number of tests which may be performed at the same time, the number of samples under examination, the number of samples waiting for examination and the number of samples in isolation.
- Provincial Sanitary and Epidemiological Station (Wojewódzka Stacja Sanitarno-Epidemiologiczna – WSSE) – reports on new positive cases (three times daily at 7:00 AM, 1:00 PM and 7:00 PM).

Additionally, depending on whether the result obtained was negative or positive, the COVID laboratory must provide information about:

- a positive result together with the patient's data to:
  - the contracting unit,
  - the dedicated COVID-19 hospital with competence over the patient's place of residence (result scan and ZLB.1 form),
  - the District Sanitary Inspector with competence over the tested person's place of residence (result scan and ZLB.1 form),
  - the Provincial Sanitary and Epidemiological Station (Powiatowa Stacja Sanitarno-Epidemiologiczna – PSSE) (ZLB.1 form).
- a negative result together with the patient's data to:
  - the contracting unit
  - the District Sanitary Inspector with competence over the tested person's place of residence (scan of the laboratory result report).

The COVID laboratory is also obliged to submit weekly reports on the number of molecular tests performed to the Provincial Sanitary Inspectorate (Wojewódzki Inspektorat Sanitarny).

If tests are reimbursed by the National Health Fund, the laboratory has to enter data and results into the EWP3 system (https://ewp3.mz.gov.pl).

Moreover, the laboratory described here must prepare everyday reports on all the results obtained in a day and on the numbers/amount of the personal protection equipment in stock for the hospital unit.

Conclusion

From March until the end of September 2020 over 5,700 tests were performed at the COVID Laboratory of the Wroclaw Comprehensive Cancer Centre to meet the hospital's needs (tests of employees and patients, including those hospitalised during the pandemic and patients before admission), which made it possible to ensure the hospital's operation in the pandemic peak as well as after some of the restrictions were lifted and has currently become a standard part of its activity. This article describes the most important aspects related to the launch and operation of a COVID laboratory at an oncology hospital. The authors hope that their experiences will facilitate the planning and implementation of SARS-CoV-2 diagnostics for new units. As there were no prior attempts of diagnosing this infection in Poland and any experiences in this area go back to mid-March 2020, the authors of this paper are open to any constructive critical remarks.

Conflict of interest: none declared

Izabela Łaczmańska
Wroclaw Comprehensive Cancer Centre
Hirszfelda Square 12
53-413 Wrocław, Poland

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