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PCR TESTIRANJE NA SARS-CoV-2: PRAKSA, PREPORUKE I NEDOUMICE

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SAŽETAK

Pandemija Kovid-19 je pred dijagnostičke laboratorije postavila nove zahteve koji su višestruko premašili postojeće kadrovske, tehničke i materijalne kapacitete. Prema važećim preporukama, osnova laboratorijske dijagnostike Kovid-19 je *Real-Time Reverse Transcription Polymerase Chain Reaction* (rtRT-PCR) kojim se detektuje prisustvo genoma SARS-CoV-2 u biološkom materijalu uzetom od pacijenta. Postupak testiranja se uslovno može podeliti u nekoliko faza: 1) postavljanje indikacija za testiranje; 2) uzimanje kliničkih uzoraka i uvođenje u evidenciju; 3) transport materijala do dijagnostičke laboratorije; 4) prijem i razvrstavanje materijala; 5) obrada uzoraka nakon prijema – priprema za rtRT-PCR; 6) rtRT-PCR; 7) saopštavanje rezultata. Jasno je da medicinske mikrobiološke službe nisu normirane za borbu protiv pandemije ili velikih epidemija. Najefikasniji način da se prevaziđe takva situacija je da se definišu timovi koji bi se uključivali u dijagnostiku kada to epidemiološka situacija nalaže. Potrebno je težiti da oprema bude unificirana na celom prostoru Republike Srbije, jer se na taj način olakšava održavanje, nabavka rezervnih delova i potrošnih materijala, a verovatno može da se umanji i nabavna cena. Reagensi za ekstrakciju i specifični reagensi za rtRT-PCR (prajmeri i probe) treba da budu standardizovani i domaćeg porekla kako bi se umanjila zavisnost od uvoza i značajno uštedeo novac. Baza podataka laboratorijskih rezultata treba da bude unapređena i organizovana na FAIR (*findability, accessibility, interoperability and reusability*) principima kako bi se maksimalno iskoristila mogućnost izvođenja širih zaključaka.

Ključne reči: Kovid-19, rtRT-PCR, preporuke eksperta

Pandemija Kovid-19

Pandemija Kovid-19 izazvana širenjem korona virusa SARS-CoV-2 (teški akutni respiratorni sindrom koronavirus 2) sa više od dvadeset miliona zaraženih i više od osam stotina hiljada preminulih je nanela neizmerne štete gotovo svim aspektima uobičajenog života u savremenom društvu (1). Pred dijagnostičke laboratorije je postavila nove zahteve koji su višestruko premašili postojeće kadrovske, tehničke i materijalne kapacitete. Prema važećim preporukama, osnova laboratorijske dijagnostike Kovid-19 je *Real-Time Reverse Transcription Polymerase Chain Reaction* (rtRT-PCR) kojim se detektuje prisustvo genoma SARS-CoV-2 u biološkom materijalu uzetom od pacijenta (2). Postupak testiranja se uslovno može podeliti u nekoliko faza: 1) postavljanje indikacija za testiranje; 2) uzimanje materijala i uvođenje u evidenciju; 3) transport materijala do dijagnostičke laboratorije; 4) prijem i razvrstavanje materijala; 5) obrada uzoraka nakon prijema – priprema za rtRT-PCR; 6) rtRT-PCR; 7) saopštavanje rezultata. Pored ovih faza koje su direktno vezane za testiranje potrebno je u celini sagledati još neke aspekte bez kojih je nemoguće sprovesti testiranje, a to su kadrovi koji izvode testove, nabavka svih potrebnih materijala (i lične zaštitne opreme) koji se koriste u testiranju.

Cilj ovog rada je da se opiše lično ikustvo stečeno kroz sve pomenute faze, kao i da se iznesu predlozi za poboljšanje postojećih procesa i neke od nedoumica.

Postavljanje indikacija za testiranje na Kovid-19

Ova faza malo zavisi od ljudstva u laboratoriji i njihov doprinos u donošenju odluka je zanemarljiv. Testiraju se osobe koje imaju kliničke simptome i znake akutne respiratorne infekcije, njihovi kontakti, lekari i drugo

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PCR TESTING FOR SARS-CoV-2: PRACTICE, RECCOMMENDATIONS AND DILEMMAS

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SUMMARY

In the Covid-19 pandemic, diagnostic laboratories have met multiple new demands, which have gone beyond the existing personnel, technical and material capacities. According to the current recommendations, the base of laboratory diagnostics for Covid-19 is a Real-Time Reverse Transcription Polymerase Chain Reaction (rtRT-PCR) test, which detects the presence of SARS-CoV-2 genome in the biological material collected from the patient. The procedure of testing can conditionally be divided into several stages: 1) setting the indications for testing; 2) collecting the clinical samples and filling in the documentation; 3) transport of the material to the diagnostic laboratory; 4) reception and classification of the material; 5) the analysis of samples after the reception – the preparation for rtRT-PCR; 6) rtRT-PCR; 7) communicating results. It is clear that medical microbiological services have not been standardized for the fight against the pandemic or great epidemics. The most efficient way to overcome such a situation is to define teams, which would take part in the diagnostics, when the epidemiological situation demanded it. It is necessary to strive to have the unified equipment on the whole territory of The Republic of Serbia, because in that way the maintenance and procurement of spare parts and consumables are made easier, and the purchasing price could possibly be lowered. Reagents for the extraction and specific reagents for rtRT-PCR (primers and probes) should be standardized and made in Serbia in order to decrease the dependence on imports and make significant money savings. The database of laboratory results should be improved and organized according to the FAIR (findability, accessibility, interoperability, and reusability) principles in order to use the possibility of making conclusions maximally.

Keywords: Covid-19, rtRT-PCR, expert's recommendations

The Covid-19 pandemic

The Covid-19 pandemic caused by the spread of coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) with more than twenty million infected people and more than eight hundred thousand deaths, has caused immeasurable harm to almost all aspects of everyday life in the contemporary society (1). Diagnostic laboratories have met new demands, which have gone beyond the existing personnel, technical and material capacities. According to the current recommendations, the base of laboratory diagnostics of Covid-19 is *Real-Time Reverse Transcription Polymerase Chain Reaction* (rtRT-PCR), which detects the presence of SARS-CoV-2 genome in the biological material collected from the patient (2). The procedure of testing can conditionally be divided into several stages: 1) setting the indications for testing; 2) collecting the clinical samples and filling in the documentation; 3) transport of the material to the diagnostic laboratory; 4) reception and classification of the material; 5) the analysis of samples after the reception – the preparation for rtRT-PCR; 6) rtRT-PCR; 7) communicating results. In addition to these stages, which are directly connected with testing, some other aspects should necessarily be taken into consideration, because without them testing cannot be conducted, and they include the personnel, who run tests, and the procurement of all the necessary materials (including the personal protective equipment), which are used during testing.

medicinsko i nemedicinsko osoblje koje je bilo u kontaktu sa obolelima ili infektivnim materijalom, službenici organa reda i pripadnici vojske koji su bili u kontaktu sa zaraženim osobama i, u zavisnosti od epidemiološke procene, osobe koje dolaze iz prekograničnih žarišta infekcije. U poslednje vreme nameće se velika potreba za testiranjem osoba koje bi želele da putuju u zemlje čija administracija zahteva negativan rtRT-PCR rezultat za prelazak državne granice. Ostaje pitanje da li bi trebalo uvesti obavezno periodično testiranje za osobe koje rade u kontaktu sa decom kao što su zaposleni u predškolskim ustanovama, osnovnim i srednjim školama, fakultetima i službama socijalne zaštite.

Uzimanje materijala i uvođenje u evidenciju

Uzimanje materijala je jednako važno kao i svaka druga faza u procesu testiranja i u njoj ima najviše prostora za unapređenje i poboljšanje organizacije. Uzorkovanje se vrši na više stotina mesta u Srbiji i, nažalost, nije uniformno što veoma otežava postupke u laboratorijskom testiranju. Najidealnije postupak treba da se realizuje na sledeći način. Po prijemu pacijenta, na mestu uzorkovanja, trebalo bi da se unese njegov matični broj u Kovid-19 bazu, da se pripremi posuda/epruveta sa nalivenim transportnim medijumom i sa zalepljenom šestoznakovnom nalepnicom i bar-kodom (jedinstvenim za čitavu zemlju) te da se imenu pacijenta u Kovid-19 bazi pridruži šestoznakovna šifra pomoću bar-kod čitača, a zatim da se opredeli u koju laboratoriju se uzorak šalje. Ovo je veoma važno, jer greška pri manuelnom unošenju (bar-koda, a zatim imena i prezimena, čak pisma kojim je ime ubačeno, JMBG-a) može da onemogući kasniju identifikaciju uzorka i prouzrokuje velika kašnjenja ili onemogući izdavanje validnih rezultata. Pogrešan izbor laboratorije u koju se uzorak upućuje veoma otežava, a u slučaju velikog opterećenja dijagnostičke laboratorije, u potpunosti onemogućava pravovremenu identifikaciju i obradu uzorka.

Uzimanje uzoraka bi trebalo da se vrši pomoću brisa od dakrona, rejona ili viskoze na tankom plastičnom štapiću sa obaveznom tačkom lomljenja, negde na 45 mm od vrha brisa. Nepostojanje tačke prekida često može voditi teškoćama prilikom unošenja i zalomljenja brisa u epruvetu te mogućoj kontaminaciji okoline. Bris bi trebao da bude uzet iz predela nazofarinksa. Praksa je, naročito u početku, bila potpuno drugačija. Uzimani su bris ždrela i nosa pomoću dva drvena štapića na koje je bila namotana vata. Takvi brisevi nisu adekvatni jer drvo može da sadrži inhibitore sledstvenih enzimskih reakcija, pamuk nije adekvatan za uzimanje materijala za pretragu na viruse PCR metodom, a mesta sa kojih su uzimani brisevi (nos i ždrelo) nisu najpogodnija za dijagnostiku SARS-CoV-2.

Važno je pomenuti i posude sa transportnim medijumom i sam transportni medijum u koji su brisevi kasnije potapaju. Idealno bi bilo da se virusni transportni medijum (VTM) 1,5-2 ml sipa u posude nalik na *Bijou* kontejnere (zapremina 5-6 ml, visine oko 50 mm i unutrašnjeg prečnika oko 15 mm sa poklopcem koji dihtuje), jer obrada uzorka iz takvih posuda izrazito ubrzava proces dalje obrade. Korišćenje posuda od 50 ml ili epruveta od 15 ml, standardnih epruveta 12 × 75 mm i raznih drugih kreativnih i iznuđenih rešenja otežava i usporava dalje postupke obrade uzoraka. Dovoljno je reći da svako zaranjanje pipete sa nastavkom u posudu čiji je zid viši od dužine nastavka nosi opasnost od kontaminacije pipete i zahteva njenu dekontaminaciju nakon svakog uzorkovanja što jako otežava rad i usporava proces dijagnostike.

Što se tiče tečnosti u koju se brisevi potapaju najbolje bi bilo da se koristi VTM koji proizvodi Torlak, jer je kompatibilan sa svim metodama dalje obrade uzorka. U idealnom slučaju *Bijou* kontejneri bi se punili na Torlaku odgovarajućom količinom VTM obeleženi nalepnicom sa šestoznakovnom šifrom i bar-kodom i zajedno sa brisevima od pogodnih materijala, na štapićima lomljivim na 45 mm od vrha, distribuirali svim mestima za uzorkovanje.

Uzorkovanje može da se vrši u prostoriji ili na otvorenom. Uzorkovanje na otvorenom je praktično, međutim nije moguće da se sprovodi u svim vremenskim uslovima. Uzorkovanje u prostorijama može da se obavlja u zdravstvenim ustanovama, ili izvan njih, na terenu. Ostaje dilema da li bi u slučaju uzorkovanja u zdravstvenim ustanovama bilo potrebno da se uzimaju ambijentalni uzorci kao kontrola uzorkovanja.

The aim of this study was to describe the personal experience, gained during the above-mentioned stages, as well as to put forward proposals for the improvement of existing processes and some dilemmas.

Setting the indications for Covid-19 testing

This stage does not depend much on the personnel in the laboratory and their contribution to decision-making is negligible. People, who have clinical symptoms and signs of acute respiratory infection, their contacts, doctors and other medical and non-medical personnel, who have been in contact with patients or infectious material, police officers and members of the army, who have been in contact with infected people, and depending on the epidemiological estimates, people who come from the cross-border focus of infection, are tested. There has been a great need recently for testing people, who would like to travel to countries, whose administration demands a negative rtRT-PCR result to cross the border. The question remains whether periodical compulsory testing should be introduced for people who work with children, that is, people who are employed in pre-school institutions, primary schools, high schools, faculties, and social protection services.

Collecting the material and filling in the documentation

Collecting the material is as important as all the other stages in the process of testing and this stage, most space is left for the progress and improvement of the organization. Collecting the samples is performed in more than a hundred places in Serbia, and unfortunately, it is not a uniform process, and therefore, it hinders the procedure of laboratory testing. The most ideal procedure should be performed in the following way: after admission, at the place of sample collection, the patient's personal identification number should be entered into the Covid-19 base, the container/test tube should be prepared with the filled transport medium and six-digit label with the barcode (unique for the whole country) should be stuck onto it, and then six-digit code with the help of barcode scanner should be added to the patient's name in the Covid-19 database, and one should choose to which laboratory the sample will be sent. This is very important because mistakes during the manual entry (of the barcode, name, surname, even the alphabet which was used to write, personal ID number) can make it impossible to identify the sample, give valid results or cause great delays. The wrong choice of the laboratory, which the sample is directed to, can be very hindering, and due to the great burden put on diagnostic laboratories, timely identification and analysis of the sample become completely impossible.

The collection of samples should be done with the swab made of dacron, rayon, viscose on a thin plastic shaft with the compulsory breakpoint, somewhere at 45 mm from the tip of the swab. The absence of this breakpoint can lead to difficulties when placing the swab into the tube and breaking it off and therefore, to the possible contamination of the environment. The swab should be collected from the region of the nasopharynx. The practice, especially at the beginning, was completely different. A throat swab and a nose swab were collected with the help of two wooden shafts with cotton buds. Such swabs are inappropriate because wood can contain the inhibitors of ensuing enzyme reactions, cotton is not an appropriate material for collecting the material for testing viruses with the help of the PCR method, and areas (throat and nose), which the swabs were collected from, are not the most suitable areas for the diagnostics of SARS-CoV-2.

It is important to mention the containers with the transport medium and the transport medium itself, which the swabs are inserted into. It would be ideal to pour the viral transport medium (VTM) 1.5-2 ml into containers similar to *Bijou* containers (volume 5-6 ml, with a height of about 50 mm and an inner diameter of about 15 mm with the lid which has good seals) because the analysis of samples from such containers accelerates the process of further analysis. The use of containers of 50 ml or test tubes of 15 ml, standard test tubes 12 × 75 mm and other creative and imposed solutions hinder and slow down further processes of sample analysis. It would be enough to say that the insertion of pipette tips into the container, whose wall is higher than the length of the pipette tip, bears the danger of pipette's contamination and it

Transport kliničkih uzoraka do dijagnostičke laboratorije

Nakon uzimanja brisa, i potapanja u transportni medijum, uzorci se čuvaju u frižideru na 2-8°C, ne duže od 24 časa, a zatim šalju u laboratoriju. Uzorci treba da budu propisno zatvoreni, svaki u zasebnoj zip-lok kesici, i da se transportuju uspravno, po mogućustvu u stalcima, najbolje u grupama od 12 uzoraka, sa složenim pratećim listama (ukoliko postoje), a obavezno sa listom svih uzoraka koja treba da bude identična otpremnici generisanoj iz Kovid-19 baze. Tokom transporta mora da se obezbedi temperatura 2-8°C što može da se postigne pomoću ručnih frižidera za transport i leda, ili toplotnih puferskih pakovanja. Prilikom transporta, frižider sa uzorcima mora da bude učvršćen na takav način da ne dođe do prevrtanja. Od momenta preuzimanja frižidera sa uzorcima, tokom transporta i predaje uzoraka vozač je dužan da se pridržava svih mera lične zaštite. U praksi, uzorci dolaze bez stalaka, često umotani u dve rukavice što veoma usporava proces prijema i razvrstavanja uzoraka u dijagnostičkim laboratorijama, a potencijalno može da izazove kontaminaciju prostora i osoblja na prijemu.

Najbolje bi bilo da se uzorci transportuju u stalcima po 12 (ukoliko ne postoje, treba ih napraviti od klirita isečenog na CNC (engl. *Computer Numerical Control*) mašinama, nije skupo, a jako olakšava razvrstavanje i obradu), uz odštampanu otpremnicu u ručnim frižiderima sa hladnim pakovanjima za višekratnu upotrebu.

Prijem i razvrstavanje kliničkih uzoraka

Prijem i razvrstavanje kliničkih uzoraka je jedna od dve faze u kojima dolazi do najvećeg zagušenja u procesu testiranja. Postupak u različitim laboratorijama se bitno razlikuje. U pojedinim laboratorijama (npr. Torlak) svaki uzorak se unosi ručno u knjigu prijema i dodeljuje mu se broj laboratorijskog protokola (flomasterom se ispisuje na posudu sa brisom), liste se uparuju sa uzorcima i dodeljuje im se isti broj, a nakon toga se uzorak šalje na dalju obradu.

U drugim laboratorijama (npr. laboratorija Vatreno oko, Beograd) uzorci i svi prateći materijali se odmah po prispeću inaktivišu toplotom temperature 60-70°C u trajanju 45-60 minuta, a zatim se pomoću bar-kod skenera praktično potvrđuju prijemi uzoraka i upoređu sa elektronskom listom uzoraka iz otpremnica kreiranih u Kovid-19 bazi, da bi se dalje slali na ekstrakciju pri čemu je šestoznakovna šifra ujedno i broj laboratorijskog protokola.

Kasnije je služba prijema na Torlaku implementirala primenu bar-kod čitača i unosila broj protokola elektronski u Excel tabelu, ali je nastavljeno pisanje laboratorijskih brojeva protokola na posudama sa transportnim medijumom i listama.

U ovoj fazi je neophodna priprema i obeležavanje potrebanog broja epruveta za dalju obradu (može da varira 1-3), kao i kriotube koja služi za dugotrajno čuvanje transportnog medijuma u zamrzivačima na -80°C. U laboratoriji Vatreno oko, prilikom izolacije je pomoću bar-kod čitača uzorak unošen u tačnu poziciju na mikrotitar ploči za automatsku izolaciju robotom *(deep-well* ploča), a sama ploča je dobijala svoj jedinstveni bar-kod koji je štampan u triplikatu (za mikrotitar ploču za alikvote uzoraka, mikrotitar ploču sa izolatima RNK i mikrotitar ploču za rtRT-PCR).

Svakako bi bilo ispravnije da uzorak odmah dobije laboratorijski broj protokola, jer je izuzetno koristan kad se dodeljuje sukcesivno na prijemu i olakšava nalaženje, identifikaciju i ponavljanje obrade uzorka u slučaju da se za tim pojavi potreba, ali nisam siguran da su liste neophodne i koja je njihova prednost u odnosu na isključivo oslanjanje na elektronsku evidenciju. Sa druge strane, vođenje knjige protokola je, osim kad bi bilo elektronsko i automatsko, suvišno, jer preopterećuje ljudstvo na prijemu i u praksi otvara mogućnost za nastanak grešaka. Laboratorijski broj protokola je izuzetno korisno obeležje uzorka u situaciji u kojoj se ne koriste bar-kod čitači jer se dodeljuje sukcesivno na prijemu i olakšava nalaženje, identifikaciju i ponavljanje obrade uzorka u slučaju da se za tim pojavi potreba.

Inaktivacija uzoraka je takođe osetljivo pitanje. Svakako, to je deo rutinske procedure u laboratorijama Vatreno oko u koje brisevi stižu u posebno formulisanom transportnom medijumu, ekstrakcija se vrši MGI reagensima, a rtRT-PCR pomoću reagensa BGI proizvođača, a razlog verovatno leži u smanjenju opasnosti

demands decontamination after each sampling, which hinders and slows down the diagnostic process.

When we consider liquids, which the swabs are inserted into, VTM, produced by "Torlak", is recommended as the best solution because it is compatible with all the methods of further sample analysis. In the ideal case, *Bijou* containers would be filled at the Institute "Torlak" with the suitable quantity of VTM and labeled with a six-digit code and barcode, and together with swabs made of appropriate materials, on shafts with the breakpoint at 45 mm from the tip, would be distributed to all places, where samples are collected.

Sampling can be performed inside or in the open space. Sampling in the open space is practical; however, it is not possible in all weather conditions. Sampling can be performed in the rooms within the health care institutions or outside of them, on the terrain. The dilemma remains whether in case of sampling in health care institutions it would be necessary to collect ambiance samples as the means of sampling control.

The transport of clinical samples to diagnostic laboratories

After swabs are collected and inserted into the transport medium, the samples are kept in the fridge at 2-8°C for no longer than 24 hours, and then they are sent to the laboratory. Samples should be properly closed, each in a separate zip lock bag, and transported in the upper position, possibly in stands, best in groups of 12 samples, with complex lists (if they exist), and obligatorily with the list of all samples which should be identical to the delivery note generated from the Covid-19 database. During transport, the temperature of 2-8°C should be provided, which could be achieved by hand fridges for the transport of ice or heat-resistant buffer packaging. During transport, the fridge with samples should be tightened in order not to be turned over. The driver has to respect all the measures of personal protection when he takes the fridge with samples, during transport, and when he hands over the fridge. In practice, samples come without stands, they are often wrapped in two gloves, and this slows down the process of reception and classification in diagnostic laboratories, and potentially this can cause the contamination of space and personnel during the reception.

It would be best to transport 12 samples in stands (if there are no stands, they should be made of clirite cut on CNC (Computer Numerical Control) machines; this is not expensive and it makes the classification and analysis easier), with the printed delivery note in hand fridges with cold packages for the repeated use.

The reception and classification of samples

The reception and classification of clinical samples is one of the two phases, when the process of testing becomes clogged. The procedure significantly differs in different laboratories. In some laboratories (e.g. "Torlak), each sample is entered manually into the book of reception and the number of laboratory protocol is assigned to it (it is written with a marker on the container with the swab), the lists are matched with the samples and the same number is assigned to them, after which the specimen is sent to further processing.

In other laboratories (e.g. laboratory "Fiery Eye", Belgrade), samples and all the materials are immediately upon reception inactivated at temperature 60-70°C lasting 45-60 minutes, and then with the help of barcode scanners the reception of samples is confirmed and they are compared with the electronic mailing lists of samples from delivery notes created in the Covid-19 database, so that they could be sent to extraction, while the six-digit code is at the same time the number of laboratory protocol.

Later, the service of reception at the Institute "Torlak" implemented the barcode scanner and entered the number of protocol electronically into the *Excel* table, but they continued to write the numbers of laboratory protocols on containers with transport medium and on the lists.

During this stage, it is necessary to prepare and mark the number of test tubes needed for further processing (it can vary from 1-3), as well as cryotube, which is used for the long-term maintenance of transport medium in freezers at -80°C. In the laboratory "Fiery Eye", during the isolation with the help of a barcode scanner, the

od infekcije u ambijentu sa više hiljada analiziranih uzoraka dnevno. Ipak, skromni paralelni eksperiment sa relativno malim brojem uzoraka (nije bilo dovoljno vremena da se testira veliki broj uzoraka paralelno) iz VTM uz lizu sa *SanSure release* reagensom i rtRT-PCR, korišćenim prema uputstvu iz *SanSure* kita, pokazao je da se nakon inaktivacije u vodenom kupatilu u trajanju od 45 min na 56°C drastično smanjuje broj pozitivnih uzoraka. To znači da je neophodno paralelni eksperiment ponoviti na većem broju poznatih uzoraka sa svakom novom vrstom transportnog medijuma, svakom novom vrstom obrade uzorka, kao i sa svakim novim kitom za detekciju (validacija metode).

Veoma je važno da se obezbedi ljudstvo za obeležavanje epruveta i njihovo slaganje u stalke kako bi se olakšala dalja obrada uzoraka. Obeležavanje epruveta mora da bude čitko, tankim flomasterima koji se teško brišu sa polipropilena, može da se vrši u prostorima koji nisu namenjeni za rad sa infektivnim materijalom i da se obeležene epruvete sukcesivno dostavljaju u laboratoriju za obradu materijala. Štampanje jedinstvenog bar-koda u više primeraka i korišćenje bar-kod čitača (za svaki sukcesivni korak obrade) u mnogome bi olakšalo datu proceduru. Prazni stalci se nakon dezinfekcije i sušenja vraćaju nazad u prostoriju gde se obeležavaju epruvete. U laboratorijama Vatreno oko se u obradi uzorka ne koriste epruvete nego *deep-well* ploče sa 96 mesta koje se jedinstveno obeležavaju što bitno olakšava postupak i gotovo u potpunosti eliminiše potrebu za obeležavanjem epruveta, osim krio-tuba.

Neophodno je preporučiti korišćenje *deep-well* ploča, isto bi bilo potrebno eksperimentalno odrediti vreme centrifugiranja kada se obrada uzoraka vrši pomoću reagenasa za lizu, jer se za ove ploče koriste *swinging bucket* rotori koji ne mogu da pruže dovoljno veliku brzinu centrifugiranja za razliku od *fixed-angle* rotora za epruvete. Razlog je lakši rad, smanjenje grešaka u obeležavanju i pozicioniranju uzorka prilikom postavljanja u rotor i vraćanja u stalak, kao i ubrzavanje rada tokom obrade korišćenjem multikanalnih pipeta. Takođe, neophodna je intenzivna obuka kadra na prijemu za rad u *Excel*-u jer sam lično imao prilike da se uverim da znanje za korišćenje tog programa, veoma blago rečeno, nezadovoljavajuće, iako, makar među mlađim laborantima to ne bi smeo da bude slučaj s obzirom da se korišćenje *Excel*-a, na mnogo višem nivou, uči u drugom razredu srednje škole.

Još jedan problem predstavljaju pritisci koji dolaze spolja da se pojedini uzorci preko reda obrade. Svi smo svesni da je nemoguće da se takva praksa u potpunosti iskoreni, ali osoblje koje razvrstava uzorke nikako ne treba da bude izloženo toj pojavi. Ukoliko postoji potreba da se iz nekog razloga neki uzorci obrade preko reda, onda je neophodno da šef ekipe identifikuje te uzorke i donese ih iz kofera na razvrstavanje i dodeljivanje broja laboratorijskog protokola. U protivnom, može da dođe do prevelikog remećenja i usporavanja procesa dijagnostike i nastanka grešaka kakve su npr. preskakanje ili dupliranje brojeva.

Obrada uzoraka i priprema za rtRT-PCR

Obrada uzoraka i priprema za rtRT-PCR može da se odvija u dva pravca. To su 1) liza sedimenta transportnog medijuma i 2) ekstrakcija nukleinskih kiselina (NK) iz transportnog medijuma. Prednost lize je brzina izvođenja (moguće i niža cena), a prednost ekstrakcije NK je čistoća uzorka koji se amplifikuje. Nezavisno od toga da li se radi liza ili ekstrakcija NK, potrebno je da se automatskom pipetom prenese zadata količina transportnog medijuma u epruvetu ili *deep-well* ploču i u krio-tubu u kojoj se čuvaju smrznuti uzorci. Veoma je važno da se posude sa brisom pre uzimanja uzorka za dalju obradu snažno promućkaju (poželjno na vorteksu) da bi se uzorak homogenizovao. Ukoliko se uzorak nalazi u uskim posudama (<25 mm unutrašnjeg prečnika) sa zidom višim od 60 mm neizostavno se javljaju komplikacije. Kontaminacija automatske pipete je izvesna, što veoma usporava rad, jer posle svakog uzorka pipeta mora da se dekontaminira i povećava se mogućnost unakrsne kontaminacije uzoraka. To može da se prevaziđe tako što se uzorak prvo prenese Pasterovom/jednokratnom transfer pipetom u krio-tubu pa se zatim odatle pipetira potrebna količina uzorka za dalju obradu. Ono što može da predstavlja problem to je da se Pasterove pipete pojedinačno pakuju što zahteva otvaranje svake pojedinačno, dovodi do velikog gubitka vremena i stvara velike količine

specimen was placed into the correct position on the microtitre plate for the automated isolation with the help of a robot (deep-well plate), and the plate itself was given a unique barcode, which was printed as a triplicate (for the microtitre plate for the aliquots of samples, microtitre plate with RNK isolates, and microtitre plate for rtRT-PCR).

Certainly, it would be more correct to assign the laboratory number of protocol to the specimen, because it is useful when it is assigned successively upon reception and it facilitates finding, identifying, and repeating the processing of specimen if the need appears. However, I am not sure whether the lists are necessary and what their advantage in comparison to electronic documentation is. On the other hand, keeping the book of protocol, except if it was electronic and automatic, is needless because it overburdens the personnel at reception, and in practice, it opens up the possibility of making mistakes. The laboratory number of the protocol is an extremely useful mark of the specimen when barcode scanners are not used because it is assigned successively upon reception and it facilitates finding, identifying, and repeating the processing of sample if it is necessary.

The inactivation of samples is also a touchy question. Certainly, it is part of the routine procedure in the laboratory "Fiery Eye", to which swabs are delivered in the specially formulated transport medium, the extraction is done with MGI reagents, while rtRT-PCR is done with the help of reagents of BGI producer, and the reason probably lies in the decrease of risk of infection in the ambiance, in which a few thousand samples are analyzed on a daily basis. However, a modest parallel experiment with a relatively small number of samples (there was not enough time to test a great number of samples in parallel) from the VTM with the *SanSure* release reagent and rtRT-PCR, used according to the instructions from the *SanSure* kit, showed that after the inactivation in the water bathroom lasting 45 minutes at 56°C drastically reduced the number of positive samples. It means that the parallel experiment should be repeated on a larger number of known samples with new types of transport medium, new types of sample processing, as well as with new detection kits (method validation).

It is important to provide personnel, who would mark the test tubes, and place them into stands, so that further processing of samples would be made easier. Test tubes should be marked legibly, with thin markers, which are hard to be erased from polypropylene. It can be done in rooms, which are not intended for work with infectious materials and marked test tubes can successively be delivered to the laboratory for sample processing. Printing the unique barcode in more copies and using the barcode scanner (for each successive step of processing) would make the given procedure easier. After disinfection and drying, empty stands are returned to the room, where test tubes are marked. In the laboratory "Fiery Eye", test tubes are not used for sample processing, but deep-well plates with 96 spaces, which are uniquely marked, which facilitates the procedure and almost completely eliminates the need to mark the test tubes, except cryotubes.

It is necessary to recommend the use of deep-well plates, although the time of centrifugation should be determined experimentally when the sample processing is done with the help of reagents for lysis, because swinging bucket rotors are used for these plates and they cannot offer sufficiently great speed of centrifugation in comparison to fixed-angle rotors for test tubes. The reason is the easier work, and the reduction of mistakes regarding marking and positioning of samples during their placement into the rotor and back to the stand, as well as faster processing with the help of multichannel pipettes. Also, it is necessary to train the personnel at reception to work in *Excel*, because I had the chance to see that knowledge about that program is unsatisfactory, although at least among younger laboratory technicians this should not be the case because the use of *Excel* is studied in the second year of high school, but at a lot higher level.

One more problem is pressure from outside to analyze some samples out of turn. We are all aware of the fact that such practice cannot be eradicated completely, but personnel, who classify the samples, should not be exposed to such occurrence. If there is a need to analyze some samples out of turn, then it is necessary that the head of that team identifies those samples and brings them from the suitcase so that they could

otpada koji se tretira kao infektivan jer se otvara u laminarnoj komori. Druga mogućnost je da se uzorak transportnog medijuma dekantuje u krio-tubu što sa sobom nosi opasnost od prosipanja uzoraka i kontaminacije zaštitne opreme i radne površine i opreme.

Brzina obrade je varirala u zavisnosti od primenjene tehnologije obrade. Moja iskustva sa *SanSure release* reagensom su takva da mogu da tvrdim da dobar laboratorijski tehničar može da obradi oko tri stotine uzoraka za radno vreme od šest sati, pod uslovom da dobija obeleženu epruvetu u koju sipa uzorak za centrifugiranje i obeleženu krio-tubu u koju sipa uzorak za čuvanje. Dalji uslov je da dobija uzorke brisa u posudama koje su nalik *Bijou* bočicama. Međutim, nisu svi uzorci bili pogodni za rad sa *SanSure release* reagensom. Određeni uzorci koji su bili prebačeni iz laboratorije Vatreno oko u Beogradu u epruvetama kineske proizvodnje nisu mogli da se obrađuju na ovaj način. Ostalo je neutvrđeno da li je to zbog toga što je transportni medijum nepogodan za lizu (sadrži SDS) pomoću *SanSure release* reagensa i rtRT-PCR sa *SanSure master mix*-om, ili je to zbog termičke inaktivacije uzoraka ili iz nekog drugog razloga. U nedostatku *SanSure* reagenasa liza uzoraka iz VTM je uspešno izvođena pomoću *Arcis* reagensa za lizu i *GeneFinder* i *DAAN* kitova za rtRT-PCR.

Ekstrakcija NK u dijagnostici Kovid-19 je vršena na više načina. Ja sam imao prilike da vršim izolaciju pomoću kolona na *Qiacube* automatskom ekstraktoru, različitim *Qiagen* kitovima sa kolonicama koje se centrifugiraju, *Vector* reagensima za manuelnu ekstrakciju, MGI reagensima (ručna ekstrakcija) i *MagMax* reagensima (ručna ekstrakcija). Zajednički imenitelj svim tim metodama je mali broj uzoraka koji mogu da se obrade (varira od 60 do 96 u toku šest časova). Svakako prednost dajem magnetnoj ekstrakciji u slučaju da je dnevno potrebno da se obradi veliki broj uzoraka u odnosu na kolone, automatsku *Qiacube* ekstrakciju i precipitaciju alkoholom (*Vector*). Zajedničko ovim metodama je i to što zahtevaju značajno višu obučenost kadra i mnogo više manuelnog rada (osim ekstrakcije na *Qiacube* aparatu). Cena ekstrakcije je značajno viša kada se koriste kolonice nego magnetne kuglice ili precipitacija alkoholom. Takođe, cena potrošnog materijala (tj. broj nastavaka sa filterom) je viša kada se radi ekstrakcija NK nego kada se radi liza.

U laboratoriji Vatreno oko izolacija NK je bazirana na automatskoj izolaciji magnetnim kuglicama MGI kitovima u MGISP-960 automatizovanom robot sistemu. Ovaj pristup omogućavao je da u procesu od 90 minuta (uključujući i dekontaminaciju aparata) budu izolovane i spremne za rtRT-PCR dve mikrotitar ploče sa uzorcima (188 uzoraka), što umnogome ubrzava proces detekcije virusa i saopštavanje rezultata.

Bilo bi dobro, a i izvodljivo je, proizvesti reagense i odgovarajuće magnetne stalke, kao i potrebnu opremu, u Srbiji, što bi umanjilo potrebu za uvoznim komponentama u ovoj fazi testiranja. Moguće bi bilo napraviti i reagense za alkoholnu precipitaciju NK u Srbiji, ali sa takvim reagensima ne bi mogla da se postigne zadovoljavajuća brzina ekstrakcije koja odgovara potrebama tokom epidemije.

Najbolje bi bilo da se koristi magnetna ekstrakcija (ručna ili automatska po mogućstvu) i da se proizvedu reagensi i prateća oprema za ručnu izolaciju u Srbiji. Adekvatno dizajnirani magnetni nosači za *deep-well* ploče bi omogućili da jedan tehničar bez preteranog napora izoluje oko četiri stotine i osamdeset uzoraka u toku radnog vremena od šest sati. Dodatna korist je što bi izolovane NK bile u ploči formata 8 × 12 bunarčića što bi olakšalo transfer uzoraka u ploču za rtRT-PCR.

Po pravilu, uzorke bi trebalo čuvati na temperaturi od -80°C. Kapaciteti takvih zamrzivača su više nego ograničeni i apsolutno nedovoljni za čuvanje tolikog broja uzoraka. Na Torlaku su oni čuvani do popunjavanja kapaciteta na -80°C, a kasnije u hladnjači na -20°C. Nedostajale su jasne procedure šta se radi sa takvim uzorcima, i takve procedure treba svakako doneti.

Izvođenja rtRT-PCR testa za dijagnozu Kovid-19

Metodologija rtRT-PCR po pravilu podrazumeva dva koraka. Prvi korak je reverzna transkripcija, a drugi korak je PCR sa detekcijom nastalog PCR produkta u realnom vremenu. Kombinovanjem enzimskog miksa koji u sebi sadrži enzime reverznu transkriptazu i DNK

be classified and that the numbers of laboratory protocol could be assigned. On the contrary, the process of diagnostics could be disturbed and slowed down and mistakes could be made, such as skipping the numbers or duplicating them.

The processing of samples and preparation for rtRT-PCR

The processing of samples and preparation for rtRT-PCR can unfold in two directions: 1) the lysis of sediment of transport medium; and 2) the extraction of nucleic acid (NA) from the transport medium. The advantage of lysis is its speed (and possibly lower price), and the advantage of NA extraction is the purity of the amplified sample. Regardless of the fact whether lysis or NA extraction is performed, the given quantity of transport medium should necessarily be transported with the automatic pipette into the test tube or deep-well plate or cryotube, in which frozen samples are kept. It is very important to shake the containers with the swab before collecting the sample for further processing (desirably on vortex) in order to homogenize the sample. If the sample is in narrow containers (<25 mm of inner diameter) with the wall higher than 60 mm complications will appear for sure. The contamination of the automatic pipette is certain, which slows down the work because the pipette has to be decontaminated after each sample, and the possibility of cross-contamination of samples increases. It can be solved by transferring the sample first with the Pasteur/single-use transfer pipette into the cryotube, and from there the necessary quantity of sample is taken with the pipette for further processing. What can be problematic is that Pasteur pipettes are packed separately, which demands the opening of each pipette, and therefore time is wasted and great amounts of waste are created and this waste is treated as infectious because it is opened in a laminated chamber. The other possibility is to decant the sample of transport medium into the cryotube, which bears the risk of spilling the sample and contamination of protective equipment, surfaces, and equipment.

The speed of processing has varied depending on the applied technology. My experience with *SanSure* release reagent is such that I can claim that a good laboratory technician can process about three hundred samples for six hours, under condition that he gets a marked test tube, which he fills with the sample for centrifugation and a marked cryotube, which is filled with the sample for keeping. The next condition is that he gets the swab samples in containers similar to Bijou bottles. However, there were some samples which were not suitable for working with SanSure release reagent. Some samples, which were transferred from the laboratory "Fiery Eye" in Belgrade in test tubes made in China, could not be processed in this way. It has remained unclear whether this happened because the transport medium was not suitable for lysis (it contains SDS) with the help of *SanSure* release reagents and rtRT-PCR with *SanSure master mix*, or it happened due to the thermal inactivation of samples or some other reason. Due to the lack of SanSure reagents, the lysis of samples from VTM was successfully done with the help of Arcis reagents for lysis and GeneFinder and DAAN kits for rtRT-PCR.

The NA extraction in the Covid-19 diagnostics was performed in several ways. I had the chance to isolate with the help of columns on *Qiacube* automatic extractor, different *Qiagen* kits with columns that are centrifugated, Vector reagents for manual extraction, MGI reagents (manual extraction), and *MagMax* reagents (manual extraction). The common denominator of all these methods is the small number of samples that can be processed (it varies from 60 to 96 for six hours). I certainly give priority to the magnetic extraction when a large number of samples have to be processed in comparison to columns, automatic *Qiacube* extraction, and ethanol precipitation (*Vector*). What these methods have in common, is the fact that they demand significantly more trained personnel and a lot more manual work (except the extraction on *Qiacube* machine). The price of extraction is significantly higher when columns are used in comparison to magnetic beads or ethanol precipitation. Also, the price of consumables (that is, the number of tips with filters) is higher when NA extraction is performed in comparison to lysis.

In the laboratory "Fiery Eye", the isolation of NA is based on the automatic isolation with magnetic beads MGI kits in the MGISP-960

zavisnu DNK polimerazu omogućeno je da se te dve reakcije odigraju sukcesivno u istoj reakcionoj smeši. Korišćenje takvih reakcionih smeša značajno smanjuju manuelni rad, potrošnju nastavaka za automatske pipete sa filterima i ubrzavaju proces testiranja. Analiza izvedena rtRT-PCR metodom po pravilu traje između 90 i 120 minuta od momenta kada se završi pipetiranje. To znači da u šestočasovnoj smeni jedan operater može na jednoj PCR mašini da finalizuje tri ciklusa sa maksimalno 94 uzoraka po ciklusu odnosno 282 uzoraka. Lako je da se izračuna da na jednoj PCR mašini može da se dnevno izda čak nešto iznad 1.100 rezultata.

Reagensi za rtRT-PCR na SARS-CoV-2

Više od dvadeset različitih testova je bilo dostupno u Srbiji za testiranje uzoraka na SARS-CoV-2 PCR metodologijom. Svi reagensi sa kojima se radilo u Srbiji su omogućavali izvođenje reverzne transkripcije i *Real-Time* PCR-a u istoj reakcionoj smeši, osim reagenasa nabavljenog od firme *Vector*, sa kojima se reakcija odigravala u dva nezavisna koraka. Primenjena metodologija je u velikoj meri smanjivala mogućnost kontaminacije opreme i prostora, jer su se nakon završetka reakcije reakcione posude bacale neotvorene. Po pravilu, svi reagensi su u sebi sadržali oligonukleotide koji su amplifikovali jednu ljudsku sekvencu (što je predstavljalo internu pozitivnu kontrolu reakcije, IPC) i oligonukleotide koji su amplifikovali 1-3 virusne sekvence. Izuzetak je test nabavljen od firme *Vector* koji je amplifikovao samo virusnu sekvencu. Zbog inherentne nepouzdanosti takvog pristupa taj test se nije radio, osim kao probni test. Najveći broj testova je odrađen sa testovima kompanija *BGI* (IPC + jedan virusni gen), *SanSure* (IPC + dva virusna gena) i *GeneFinder* (IPC + tri virusna gena). Iako potreba da se na nivou države radi sa jednim testom možda deluje kao ograničavanje slobode tržišta, jasno je da bi korišćenje jednog testa na celoj teritoriji države imalo niz prednosti. Na prvom mestu, neizmerno bi se olakšala i ujednačila interpretacija rezultata na nacionalnom nivou, verovatno bi mogla da se postigne niža cena prilikom nabavke reagenasa uzimajući u obzir veće količine koje bi se naručile, bitno bi se povećala mogućnost poređenja efikasnosti laboratorija i olakšala fluktuacija kadra između različitih laboratorija.

Vredi napomenuti da ni za jedan od korišćenih testova nisu bile poznate sekvence i koncentracije oligonukleotida koje su ulazile u njihov sastav. Takva praksa proizvođača nije nova, ali ostaje pitanje da li Srbija kao država treba da pristane na takvu praksu. Pogotovo zbog toga što sinteza oligonukleotida nije naročito napredna tehnologija (ja sam devedesetih godina prošlog veka naručivao i radio PCR sa oligonukleotidima koji su sintetisani u tadašnjem Centru za genetski inženjering), niti kapaciteti za sintezu oligonukleotida za potrebe pandemijskog testiranja zahtevaju nabavku jako skupe opreme ili angažuju veliki broj ljudi. Dizajniranjem, sintezom i obeležavanjem oligonukleotida u Srbiji dobili bi mogućnost da proizvodimo sopstvene specifične testove, kako za Kovid-19, tako i za druge infektivne i mnoge neinfektivne bolesti.

Finalizacija reakcione smeše i plastika za rtRT-PCR na SARS-CoV-2

I u ovoj fazi testiranja su postojale velike razlike među laboratorijama. U Nacionalnoj referentnoj laboratoriji je uzorak (VTM) pipetiran direktno u PCR ploču u kojoj se nalazio *SanSure release* reagens, a zatim *SanSure master mix*. U laboratorijama Vatreno oko je uziman alikvot ekstrahovanih NK iz ploče i pipetiran automatizovanim sistemom direktno u PCR ploču sa prethodno pipetiranim master miksom. Na Torlaku je pipetiranje master miksa u ploču vršeno automatskom stanicom za pipetiranje *EpMotion 5070*, a uzorci su se iz pojedinačnih epruveta dodavali u master miks. Ostaje pitanje kako se radilo u drugim laboratorijama. U laboratorijama u kojima je obrađeno najviše uzoraka (Torlak, Vatreno oko Beograd i Nacionalna referentna laboratorija) rtRT-PCR reakcija se po pravilu izvodila u pločama. U pojedinim laboratorijama se rtRT-PCR izvodio u stripovima. Korišćenje ploča donosi nesumnjive prednosti, jer otvaranje i zatvaranje stripova povećava mogućnost kontaminacije i treba ga izbegavati. Isplativije je da se iskoristi samo četvrtina ploče nego da se rizikuje kontaminacija jednog uzorka. Utisak koji sam stekao je da se u radu nedovoljno koriste multikanalne pipete, delom zbog objektivnih okolnosti (neadekvatna

automated robotic system. This approach made it possible for 90 minutes (including the apparatus decontamination) to isolate and prepare two microtitre plates with samples (188 samples) for rtRT-PCR, which makes the process of detecting the virus and communicating results faster.

It would be good, and it is feasible, to make reagents and appropriate magnetic stands, as well as all the necessary equipment in Serbia, which would reduce the need for imported components in this stage of testing. It would be possible to make reagents for the ethanol precipitation of NA in Serbia, but with such reagents the satisfactory speed of extraction, which suits the needs during the epidemic, could not be achieved.

It would be best to use the magnetic extraction (manual or possibly automatic) and to produce the reagents and equipment for manual isolation in Serbia. Appropriately designed magnetic carriers for deep-well plates would enable a technician to isolate without much effort about four hundred and eighty samples during his shift of six hours. An additional benefit is that the isolated NA would be in the plate format 8 ×12 wells, which would make the transfer easier into the plate for rtRt-PCR.

As a rule, samples should be stored at temperature -80°C. The capacities of such freezers are more than limited and absolutely insufficient for storing such a large number of samples. At the Institute "Torlak" they were stored until capacities were filled at temperature -80°C, and then in the cold storage room at -20°C. Clear procedures on what to do with such samples were missing, and such procedures should certainly be regulated.

Running the rtRT-PCR test for the diagnosis of Covid-19

The methodology of rtRT-PCR, as a rule, includes two steps. The first step is the reverse transcription, and the second step is PCR with the detection of a PCR product in real-time. By combining the enzyme mix, which contains the enzymes reverse transcriptase and RNA dependent RNA polymerase, these two reactions could happen successively in the same reaction mixture. The use of such reactive mixtures significantly reduces the manual work, consumption of tips for automatic pipettes with filters, and makes the testing process faster. The analysis performed with rtRT-PCR method lasts between 90 and 120 minutes from the moment when the pipetting is finished. This means that during the shift of six hours one operator can finish three cycles of maximally 94 samples for one cycle, that is, 282 samples on one PCR machine. It can easily be calculated that more than 1.100 results can be issued on one PCR machine on a daily basis.

SARS-CoV-2 reagents for rtRT-PCR

More than twenty different tests have been available in Serbia for diagnostic testing for SARS-CoV-2 with the PCR method. All reagents, which have been used in Serbia, enabled running the reverse transcription and Real-Time PCR in the same reaction mixture, except in the case of reagents purchased from Vector, when the reaction was performed in two separate steps. The applied methodology reduced, to the great extent, the possibility of contamination of equipment and space because the closed containers were thrown away after the reaction. As a rule, all reagents contained oligonucleotides, which amplified one human sequence (which presented the internal positive reaction control, IPC) and oligonucleotides, which amplified 1-3 virus sequences. One exception is the test of *Vector* company, which amplified only the virus sequence. Due to the inherent unreliability of such an approach, the test was conducted only as an experimental test. The largest number of tests was conducted with *BGI* tests (IPC + one viral gene), *SanSure* tests (IPC + two viral genes), and *Gene Finder* (IPC + three viral genes). Although the need to use only one test in the whole country seems like a limitation of the freedom of the market, it is clear that the use of one test on the territory of the whole country would have its advantages. Firstly, it would standardize and facilitate the interpretation of results at the national level, and possibly lower the price because larger quantities would be ordered. The possibility of comparing the efficiency of laboratories would be increased and the fluctuation of personnel between different laboratories would be made easier.

geometrija stalaka ili nedostatak multikanalnih pipeta koje pokrivaju opseg 5-50 µl), a delom zbog subjektivnih razloga kao što su neiskustvo i nesigurnost u radu sa multikanalnim pipetama. To je velika šteta, jer korišćenje multikanalnih pipeta bitno ubrzava rad i umanjuje mogućnost greške.

PCR mašine

PCR mašine koje sam imao prilike da vidim u laboratorijama koje sam obišao su po pravilu imale dovoljan broj detektora za pokrivanje svih fluorohroma (ili njihovih spektralnih analoga) kojima su bili obeležene specifične probe. Sve PCR mašine imale su standardne blokove formata 8 × 12. Prostim računajem kapaciteta PCR mašine i dnevnih potreba testiranja u Srbiji lako se izračuna da petnaestak standardnih PCR mašina koje bi bile namenjene samo testiranju na SARS-CoV-2 uz pravilno korišćenje mogu da podmire potrebe cele Srbije, jer ni u jednom trenutku broj urađenih PCR testova nije prelazio deset hiljada. Tokom proleća 2020. godine je nabavljen određeni broj PCR mašina i tražen značajan broj novih PCR mašina i sve su bile sa standardnim blokom 8 × 12 pozicija. Svakako vredelo bi razmisliti o nabavci mašina sa izmenjivim blokom (npr. *ABI QuantStudio 6*) pri čemu bi se nabavio i termoblok formata 16 × 24 mesta, jer se tako povećava broj analiziranih uzoraka u jednom ciklusu četiri puta (384 naspram 96). Pri tome se i zapremine korišćenih reagenasa umanjuju što automatski umanjuje cenu testiranja. Smanjenje zapremine reagenasa ne bi umanjilo senzitivnost testiranja nakon optimizacija procesa obrade uzoraka i reakcionih smeša.

Ono što treba imati u vidu je i mali eksperiment koji je izvršen tokom prethodnih meseci rada na dijagnostici SARS-CoV-2 virusa, koji je, koristeći iste uzorke, odnosno iste izolate paralelno, pokazao da RT-PCR mašine različitih proizvođača imaju i različitu senzitivnost koja se ogledala u razlici čak i do 5 podeoka vrednosti Ct (engl. *Cycle threshold value*). Ovakva činjenica ima za posledicu da u zonama male koncentracije virusa detekcija targeta jednom RT-PCR mašinom može dati dijametralno suprotan rezultat od detekcije drugom, te da bi trebalo izvršiti dodatne analize i dati preporuke i u tom pogledu.

Programiranje PCR mašina za testiranje na SARS-CoV-2 metodom rtRT-PCR

Svi reagensi za rtRT-PCR testiranje na SARS-CoV-2 su imali jasno napisana uputstva za programiranje temperaturnih profila reakcije, u smislu definisanih temperatura i broja ciklusa koji su ponavljani. Međutim, ni u jednom uputstvu nisu definisane brzine dostizanja temperatura (ramp rate) što predstavlja važan PCR parametar i može da ima uticaj na ishod testiranja i reproducibilnost rezultata. Manuelno unošenje oznaka uzoraka u softver koji kontroliše mašinu je spor proces koji u sebi nosi veliku mogućnost greške. Iz tog razloga je najbolje koristiti jednostavne *Excel* templejte koji sa jedne strane olakšavaju unos, a sa druge strane olakšavaju saopštavanje rezultata. Odgovarajući *Excel* templejti su primenjivani u laboratorijama Torlak i Vatreno oko Beograd. Kreiranje tabele, odnosno liste za učitavanje brojeva uzoraka, je na Torlaku rešeno tako što se koristila *autofill* komanda u *Excel*-u (kada je god to bilo moguće), dok je u Vatrenom oku Beograd jednostavno kopiran plan ploče za ekstrakciju RNK prethodno unet u *Excel* pomoću bar-kod čitača. Moj utisak je da su se u ostalim laboratorijama koje sam obišao oznake uzoraka u softver unosile ručno tokom programiranja mašine.

Interpretacija rezultata rtRT-PCR testova na SARS-CoV-2

Interpretacija dobijenih rezultata je značajno varirala što je logična posledica korišćenja različitih testova. Najlakša interpretacija rezultata je bila kada su korišćeni testovi sa samo jednom ciljnom virusnom sekvencom (npr. *BGI*). I preporuke za tumačenje rezultata su se menjale tako da je u najnovijim protokolima preporučeno da se rezultat izdaje kao pozitivan i kada je samo jedan virusni gen bio pozitivan (najčešće N gen). Diskusije i razmena iskustava između rukovodilaca u različitim laboratorijama bi bile izuzetno korisne i doprinele bi podizanju kvaliteta interpretacije rezultata. Na žalost, nije ustanovljena praksa da se rukovodioci laboratorija periodično sastaju i diskutuju o interpretaciji rezultata, prvenstveno zbog izuzetnog napora koji je ulagan da se dijagnostika održi u potrebnom obimu, razuđenosti laboratorija i smenskog rada.

We should mention that sequences and concentration of oligonucleotides were not known for these tests. Such a manufacturer's practice is not new, but the question remains whether Serbia should accept such practice. Especially because the synthesis of oligonucleotides is not particularly advance technology (in the 1990s I ordered and conducted PCR with oligonucleotides, whose synthesis was performed in the Center for Genetic Engineering), and because capacities for the synthesis of oligonucleotides for the testing during pandemic demand very expensive equipment and engage a lot of people. If oligonucleotides were designed, marked, and synthesized in Serbia, we could make our own specific tests for Covid-19, as well as for infectious and many non-infectious diseases.

The finalization of reaction mixture and plastic for SARS-CoV-2 rtRT-PCR

In this stage of testing, there were great differences between laboratories. In the National Reference Laboratory, the sample (VTM) was placed with a pipette directly into the PCR plate, in which *SanSure* release reagent was, and then *SanSure* master mix. In the laboratory "Fiery Eye" the aliquot of extracted NA was taken from the plate and placed with the automated pipette directly into the PCR plate with a master mix, while the pipetting of master mix was previously conducted. At the Institute "Torlak", the pipetting of the master mix into the plate was conducted with the automated station for pipetting EpMotion 5070, while samples were added into the master mix from separate test tubes. The question remains how the other laboratories worked. In laboratories, in which the largest number of samples were analyzed (Torlak, Fiery Eye in Belgrade, The National Reference Laboratory), rtRT-PCR reaction was conducted in plates. In some laboratories, rtRT-PCR was conducted in strips. The use of plates has advantages, because the opening and closing of strips increase the risk of contamination and it should be avoided. It is worthwhile to use only one-fourth of the plate than to risk the contamination of the whole sample. My impression is that multichannel pipettes are not used sufficiently, partly due to objective circumstances (inadequate geometry of the stand or lack of multichannel pipettes which cover the range from 5-50 µl), and partly due to subjective reasons, such as inexperience and insecurity while working with multichannel pipettes. It is a great pity because the use of multichannel pipettes significantly makes the work faster and diminishes the possibility of mistakes.

PCR machines

PCR machines, which I had a chance to see in laboratories, had a sufficient number of detectors for covering all fluorochromes (or their spectral analogs) with which specific probes were marked. All PCR machines had standard block format 8 x 12. By simple calculation of the capacity of PCR machine and daily needs for testing in Serbia, it can easily be calculated that about fifteen standard PCR machines, which would be intended only for SARS-CoV-2 testing, would be sufficient for the needs of the whole Serbia, because the number of conducted tests never exceeded 10 thousand. In the spring of 2020, a number of PCR machines were purchased and a significant number of new PCR machines were asked for and they were all with the standard block 8 × 12 positions. It would be worthwhile to consider the purchasing of machines with the changeable block (e.g. ABI QuantStudio 6), as well as the thermal block in the format 16 × 24 places because the number of analyzed samples in the one cycle would be increased fourfold (384 in comparison to 96). Also, the volumes of reagents are reduced, which automatically lowers the price of testing. The reduction of the reagents' volume would not diminish the sensitivity of testing after the optimization of the processing of samples and reactive mixtures.

What one should have in mind is a little experiment, which was conducted during the previous months while working on the diagnostics of the SARS-CoV-2. The same samples, that is, the same isolates were used in parallel and it was shown that RT-PCR machines of different manufacturers have different sensitivity, which was reflected in the difference of even 5 cycles threshold value. This results in the fact that in the zones of a small concentration of virus, the detection of a target with one PCR machine can give a completely different result

Saopštavanje rezultata rtRT-PCR testova na SARS-CoV-2

Saopštavanje rezultata je takođe nosilo sa sobom dozu izazova i evoluiralo je u periodu između marta i avgusta. Na samom početku epidemije na Torlaku su rezultati ručno upisivani u protokol laboratorije, a zatim su izdavani odštampani rezultati koji su pripremani za štampu u *Word*-u nakon čega su potpisivani i pečatirani. Rezultati su krajem marta počeli da se unose u Kovid-19 bazu podataka, međutim nije jenjavao pritisak za izdavanjem štampanih rezultata (potvrda) za pojedinačna lica. Pored toga, zdravstvene ustanove i zavodi za javno zdravlje su zahtevali i zbirne tabele sa rezultatima za sve uzorke koje su poslali određenog dana. Takva praksa je dovodila do preteranog iscrpljivanja kadra koji je radio na izdavanju rezultata. Tokom aprila i maja Kovid-19 baza je u nekoliko navrata usavršavana pa su se tokom unošenja pojedinačnih rezultata pojedina polja automatski popunjavala, a omogućen je i unos više rezultata iz pripremljene *Excel* tabele. Tu opciju je koristila laboratorija Vatreno oko Beograd od početka rada u aprilu, Torlak od juna, a u ostalim laboratorijama koje sam obišao te pogodnosti nisu korišćene.

Kovid-19 baza je bitno evoluirala od marta do danas, ali još uvek postoji dosta prostora za unapređenje. Pretraživanje baze je omogućeno po određenom broju kriterijuma koji su vezani za lice, ali nikako nije omogućeno pretraživanje i filtriranje po svim poljima koja se popunjavaju u bazi ili po unetim vrednostima i nisu ispunjeni FAIR principi (*findability, accessibility, interoperability and reusability*) (3). Jasno je da nije bilo vremena i resursa da se napravi takva baza podataka, ali je to nešto čemu treba da se stremi. Za svaki uneti uzorak bi trebalo da postoje podaci sa kojim reagensima je obrađivan i amplifikovan i koje su bile Ct vrednosti IPC i virusnih gena. U idealnom slučaju u bazu bi se unosili i meta podaci kao što je izvorni fajl sa PCR mašine i fotografija ploče pre i nakon PCR-a na kojima bi se videle oznake eksperimenta i nivoi tečnosti i ti podaci bili bi povezani (linkovani) sa uzorkom. U takvom slučaju bi interpretacija rtRT-PCR rezultata mogla da se obavlja i izvan laboratorije. Dodatno, moglo bi da bude korisno otvaranje mogućnosti geolociranja mesta boravišta i prebivališta pacijenta, kao i mesta uzorkovanja i obrade uzoraka.

Na osnovu ovih podaka mogle bi da se dobijaju sledeće informacije: 1) koliko je osoba laboratorijski testirano; 2) koliko je osoba testirano rtRT-PCR metodom; 2a) koliko je od tih testova bilo pozitivnih (broj i procenat); 2b) koliko je od tih testova bilo prvo testiranje neke osobe; 2c) koliko je od tih testova bilo pozitivnih (broj i procenat); 3) koliko je urađeno seroloških testova; 3a) koliko je urađeno imunohromatografskih testova; 3b) koliko je urađeno enzimskih testova; 3c) kolika je seroprevalencija među testiranima tog dana; 3d) kolika je kumulativna seroprevalencija među testiranima; 4) koliko je urađeno imunohromatografskih testova na antigen. Takva struktura podataka bi mnogo jasnije potkrepila izjave zvaničnika o ozbiljnosti epidemiološke situacije.

Kadrovske potrebe za testiranje kliničkih uzoraka na SARS-CoV-2

Već u trećoj dekadi marta postalo je jasno da postojeći kadrovski kapaciteti laboratorije za testiranje na Torlaku nisu dovoljni za potrebe Srbije za dijagnostiku Kovid-19. Razlozi su brojni, od neadekvatnih materijalno tehničkih uslova do nedostatka ljudstva. Nedostatak ljudstva je delimično posledica nedovoljnog broja zaposlenih sa odgovarajućim kvalifikacijama za učešće u dijagnostici, zadržavanje određenog broja koji su raspoređeni na dijagnostiku u karantinu i nemogućnosti povlačenja zaposlenih u ostalim radnim jedinicama, takođe zbog karantina. Analiza na SARS-CoV-2 jeste deo panela mikrobioloških analiza, i kao takva trebala bi da bude pod jurisdikcijom specijalista mikrobiologije sa parazitologijom. Međutim, po svojoj suštini, sve analize koje se rade u dijagnostici Kovid-19 su ili molekularne tehnike (kao npr. rtRT-PCR) ili antigen-antitelo reakcije. Ta činjenica opravdava učešće drugih specijalista laboratorijskih grana medicine i farmacije, ali i drugih profila kojima su te tehnologije bliske i koji ih koriste u svakodnevnom radu npr. veterinari, molekularni biolozi i drugi. Potpuno je jasno da većinu testova nisu uradili i većinu rezultata nisu izdali specijalisti mikrobiologije sa parazitologijom. Moj utisak je bio da je ta činjenica ostavila gorak utisak među kolegama mikrobiolozima, ali činjenica pred kojom ne smemo zatvarati oči je da službe mikrobiologije u

in comparison to the detection with another machine, and therefore, additional analyses should be done and recommendations should be made.

Programming the PCR machines for SARS-CoV-2 testing with the rtRT-PCR method

All reagents for rtRT-PCR testing for SARS-CoV-2 had clearly written instructions for programming the temperature profiles of reaction, in the sense of defined temperatures and the number of cycles which were repeated. However, the ramp rate was not defined in the instructions, which is an important parameter of PCR and can influence the outcome of testing and the reproducibility of results. The manual entry of samples into the software, which controls the machine, is a slow process that has a great possibility of mistakes. Due to that reason, it is best to use *Excel* templates, which on the one hand facilitate the entry, and on the other communicating the results. Appropriate *Excel* templates were applied in the laboratories "Torlak" and "Fiery Eye", Belgrade. Creating the table, that is, the list for the numbers of samples was solved at the Institute "Torlak" with the autofill command in *Excel* (whenever it was possible), whereas in "Fiery Eye" the plan of a plate for the extraction of RNA was copied and previously entered into *Excel* with the barcode scanner. My impression is that in other laboratories, which I have visited, the samples were entered manually into the software during the machine programming.

The interpretation of results of rtRT-PCR tests for SARS-CoV-2

The interpretation of gained results varied significantly, which is the logical consequence of using different tests. The easiest interpretation of results was when tests with one target viral sequence (e.g. *BGI*) were used. Recommendations for the interpretation of results have changed, and in the most recent protocols, it has been recommended that the result is issued as positive when even one viral gene was positive (most frequently N gene). Discussion and exchange of experience among heads of different laboratories would be extremely useful and they would contribute to the better quality interpretation of results. Unfortunately, the practice was not established that the heads of laboratories periodically meet and discuss the interpretation of results, primarily due to the extreme efforts to maintain the scope of the necessary diagnostic, the distance between laboratories, and working in shifts.

Communicating results of rtRT-PCR tests for SARS-CoV-2

Communicating results has been challenging and it evolved from March to August. At the beginning of this epidemic, at the Institute "Torlak", results were manually written into the protocol of laboratory and then printed results were issued. They were prepared in Word, and after printing signed and stamped. At the end of March, they started to enter the results into the Covid-19 database, however, the pressure to issue printed results (findings) to individuals did not stop. In addition to that, health care institutions and public health institutes demanded collective tables with results for all samples, which they sent on that day. Such practice led to the overburdening of personnel, who issued results. During April and May, the Covid-19 database was a few times improved, so during the entry of individual results certain fields were automatically filled in, and the entry of more results from the prepared *Excel* table was made possible. That option has been used in the laboratory "Fiery Eye", Belgrade since April, at the Institute "Torlak" since June and in other laboratories, which I have visited, this convenience has not been used.

The Covid-19 database has significantly evolved since March, but still further improvements can be made. Searching the database was possible according to the criteria connected with persons, but searching and filtering by all fields in the database or by entered values were not possible, and FAIR principles were not fulfilled (*findability, accessibility, interoperability, and reusability*) (3). It is clear that there was not enough time and resources to make such a database, but it is something we should strive to. For each sample, data should be entered about reagents, with which it was processed and amplified, about Ct values and viral genes. Ideally, the meta-data would be entered into the database, such as the original file from the PCR

sekundarnoj i tercijarnoj zdravstvenoj zaštiti, kao ni po regionalnim zavodima za zaštitu zdravlja, nisu kadrovski normirane, niti je ljudstvo obučeno i spremno da iznese teret jednog ovako masovnog testiranja. Neizmeran doprinos u održavanju dijagnostike u Beogradu i drugim univerzitetskim centrima su dali zaposleni sa različitih fakulteta i naučnih instituta koji su činili okosnicu visoko kvalifikovanog ljudstva angažovanog u dijagnostici Kovid-19 i koji su radili bez ikakve naknade. Pored toga, veliki teret u dijagnostici u Šumadiji i zapadnoj Srbiji i južnoj i istočnoj Srbiji izneli su veterinarski specijalistički instituti u Kraljevu, Šapcu i Nišu koji su učestvovali u testiranju, takođe, radeći bez ikakve naknade i često trošeći sopstvene potrošne materijale.

Zaključak

Jasno je da medicinske mikrobiološke službe nisu normirane za borbu protiv pandemije ili velikih epidemija. Najefikasniji način da se prevaziđe takva situacija je da se definišu timovi koji bi se uključivali u dijagnostiku kada to epidemiološka situacija nalaže. Timovi bi trebali da budu sastavljeni po regionalnom principu i da sadrže 2-3 tehničara i 2-3 visoko kvalifikovana člana od kojih bi makar jedan član morao da bude mikrobiolog. Tehničare bi trebalo regrutovati iz raznih zdravstvenih i drugih laboratorija, a ne isključivo iz mikrobioloških laboratorija (npr. sanitarni tehničari). Visoko kvalifikovani kadar bi se regrutovao iz istraživačkih i dijagnostičkih laboratorija. Poželjno bi bilo da u svakom timu bude jedan administrativni radnik (administracija i logistika) i jedan IT stručnjak za komunikaciju i podršku. Takav tim bi mogao da uzorkuje, obradi i izda rezultate za oko dve stotine i sedamdeset osoba na jednoj standardnoj PCR mašini u šestočasovnoj smeni ili za oko sto osamdeset osoba za četiri sata, u zavisnosti od potreba. Takvi timovi ne bi iziskivali novo zapošljavanje i hipertrofisanje postojećih struktura van epidemije, a tokom epidemije bi mogli brzo da se angažuju i uključe u dijagnostiku. Bilo bi potrebno da se organizuju periodično i okupljanja članova tima, kao i da se sprovode obuke i vežbe za kriznu situaciju. Takođe, potrebno bi bilo da postoji između vođa timova periodična komunikacija van perioda epidemije, a svakodnevna tokom epidemije.

Potrebno je težiti da oprema bude unificirana na celom prostoru Republike Srbije jer se na taj način olakšava održavanje, nabavka rezervnih delova i potrošnih materijala, a verovatno može da se umanji i nabavna cena. Ovakav pristup možda nije u skladu sa slobodama tržišta, ali u kriznim situacijama bi bio izuzetno isplativ. Reagensi za ekstrakciju i specifični reagensi za rtRT-PCR (prajmeri i probe) trebali bi da budu standardizovani i domaćeg porekla kako bi se umanjila zavisnost od uvoza i značajno uštedeo novac. Pored toga, tako bi bila olakšana mobilnost kadra između laboratorija, olakšana i uniformisana interpretacija rezultata i merljiva efikasnost rada u laboratorijama.

Baza podataka bi trebalo da bude unapređena i organizovana na FAIR principima kako bi se maksimalno iskoristila mogućnost izvođenja širih zaključaka.

Literatura

1. WHO. Coronavirus disease 2019 (COVID-19) situation reports. [Internet] Dostupno na: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports [Pristupljeno 31.08.2020.]
2. Caruana G, Croxatto A, Coste AT, Opota O, F. Lamoth F, Jaton K, Greub G. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. Clin Microbiol Infect 2020;26 (9):1178-1182.
3. Wilkinson MD, Dumontier M, Jan Aalbersberg IJ, Appleton G, Axton M, Baak A, et al. 'The FAIR Guiding Principles for Scientific Data Management and Stewardship'. Scientific Data 3, 2016: 160018. Available at: https://doi.org/10.1038/sdata.2016.18.

machine and the photograph of the plate before and after PCR, which would show the sign of the experiment and the level of liquid and those data would be linked to the sample. In that case, the interpretation of rtRT-PCR results could be conducted out of the laboratory. In addition to that, the possibility to locate the patient's place of residence would be useful, as well as the place of sampling and sample processing.

According to these data, the following information could be gained: 1) how many people were tested in the laboratory; 2) how many people were tested with the rtRT-PCR method; 2a) how many of these tests were positive (number and percentage); 2b) how many of these were the first tests; 2c) how many of these tests were positive (number and percentage); 3) how many serology tests were conducted; 3a) how many immunochromatographic tests were conducted; 3b) how many enzyme tests were conducted; 3c) how high the seroprevalence was; 3d) how high the cumulative seroprevalence was; 4) how many immunochromatographic tests were conducted for the antigen. Such a structure of data would clearly support the statements of officials about the seriousness of the situation.

Personnel requirements for testing of clinical samples for SARS-CoV-2

In the third decade of March, it became clear that the existing personnel capacities of the laboratory at the Institute "Torlak" were not sufficient for the needs of Serbia for the diagnostics of Covid-19. The reasons are numerous, from the inadequate material-technical conditions to the lack of personnel. The lack of personnel is partly the consequence of the insufficient number of employees with appropriate qualifications, who could participate in the diagnostics, keeping some of them in the quarantine and inability to call the employees from other departments, also due to quarantine. The analysis of SARS-CoV-2 is part of the panel of microbiological analysis, and as such, it should be under the jurisdiction of specialists of microbiology with parasitology. However, in its essence, all analyses, which are conducted in Covid-19 diagnostics, are molecular techniques (such as rtRT-PCR) or antigen-antibody reaction. That fact justifies the participation of other specialists of laboratory branches of medicine and pharmacy, or the other profiles, close to those technologies who use them in everyday work, for example, veterinarians, molecular biologists, and others. It is completely clear that the majority of tests and the majority of results were not conducted or issued by the specialists of microbiology with parasitology. My impression is that this fact was a bitter blow for microbiologists, but we should not neglect the fact that microbiological departments in the secondary and tertiary health care institutions, as well as in public health institutes, are not standardized regarding personnel, and they are not trained and prepared to take the burden of such mass testing. The immeasurable contributions to the maintenance of diagnostics in Belgrade and other university centers were made by the employees from different faculties and scientific institutes, who made the key integral part of highly-qualified personnel engaged in the diagnostics of Covid-19 and who worked with no recompense. In addition to that, a great burden of the diagnostics in the Sumadija and western Serbia, the southern and eastern Serbia was carried out by veterinarian specialist institutes in Kraljevo, Sabac, and Nis, which took part in testing, as well, working with no recompense and often spending their own consumables.

Conclusion

It is clear that medical microbiological services are not standardized for the fight against the pandemic or big epidemics. The most efficient way to overcome such a situation is to define teams, which would take part in the diagnostics when the epidemiological situation orders that. Teams should be made according to the regional principle and they should include 2-3 technicians and 2-3 highly-qualified members, and one of them should necessarily be a microbiologist. Technicians should be recruited from various health care and other laboratories, and not solely from microbiological laboratories (e.g. sanitary technicians). Highly-qualified cadre would be recruited from the research and diagnostic laboratories. It would be desirable that each team has one administrative worker (administration and logistics) and one IT professional for communication and support.

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Such a team could collect samples, process, and issue results for about two hundred and seventy people on one standard PCR machine during the shift of six hours or for about one hundred and eighty people for four hours, depending on the needs. Such teams would not demand new employment and hypertrophy of the existing structures out of the epidemic, and during epidemic, they could quickly be engaged and included in the diagnostics. It would be necessary to organize periodic meetings for team members, as well as training and practice for the crisis situation. Also, it is necessary that heads of teams communicate periodically out of the epidemic, and on a daily basis during the epidemic.

The equipment should be unified on the whole territory of the Republic of Serbia because in that way, the maintenance is made easier, as well as the procurement of spare parts and consumables, and possibly the purchasing price can be lowered. Such an approach may not be in accordance with the freedom of the market, but during the period of crisis, it would be extremely profitable. Reagents for the extraction and specific reagents for rtRT-PCR (primers and probes) should be standardized and made in Serbia so that the dependence on import would be reduced and money savings would be significant. In addition to that, the mobility of cadre between laboratories would be made easier, the interpretation of results would be uniform and easier and the efficiency of work in laboratories would be measurable.

The database should be improved and organized according to the FAIR principles so that the possibility of making wider conclusions would be maximally used.

Literature

1. WHO. Coronavirus disease 2019 (COVID-19) situation reports. [Internet] Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports [Accessed 3rd August 2020]
2. Caruana G, Croxatto A, Coste AT, Opota O, F. Lamoth F, Jaton K, Greub G. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. Clin Microbiol Infect 2020; 26 (9):1178-1182.
3. Wilkinson MD, Dumontier M, Jan Aalbersberg IJ, Appleton G, Axton M, Baak A, et al. 'The FAIR Guiding Principles for Scientific Data Management and Stewardship'. Scientific Data 3, 2016: 160018. Available at: https://doi.org/10.1038/sdata.2016.18.

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