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SARS-CoV-2 molecular diagnostics

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Summary

The new coronavirus — SARS-CoV-2 infections have recently become the main epidemiological and clinical challenge worldwide (Poland included). This article is based on the presentation entitled "Hematology and transfusion medicine and COVID-19" given/conferred during the webinar in May 2020. The aim was to introduce the procedure for diagnosing the new coronavirus infection. The procedure highlights the aspects of the pre-analytical and analytical phase that are critical for the quality of the performed tests and affected by both the commissioning and test-performing personnel. On the basis of current literature, further recommendations for the improvement of molecular diagnostics of SARS-CoV-2 are also presented.

Key words: SARS-CoV-2, RT-PCR, diagnosis

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Introduction

This article is based on *"Hematology and transfusion medicine and COVID-19"* — a presentation given/conferred during the webinar which took place on May 28, 2020. The text has been supplemented with a number of more detailed information on SARS-CoV-2 RNA detection tests. The aim of the article was to bring the reader closer to the diagnostic procedure for the new coronavirus infection. It does not however go into details of the principles of optimal performance at all stages of laboratory testing — these are set forward in both national and international recommendations [1–3]. The authors draw attention to the critical moments of the pre-analytical and analytical testing phase that are crucial for the quality of the performed tests and are affected both by the test ordering and test-performing personnel. Further recommendations for the improvement of molecular diagnostics of SARS-CoV-2 are also presented, as based on contemporary literature.

Why is the diagnosis of the new coronavirus infection of such significance for hematology? Mainly because hematological clinics and out patients treatment centers take in patients with weaker immune system from neoplastic therapy, hematopoietic cell transplants as well as patients with immunodeficiency due to other underlying medical conditions. These patients are at increased/high risk of severe illness from the virus that causes covid-19 [4].

Molecular diagnostics for covid-19

Molecular diagnostics is fundamental for diagnosis of confirmed SARS-CoV-2 infection. According to national and international recommendations as well as literature references the reliability of test results depends on the type of biological material and collection method [1–3].

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SARS-CoV-2 RNA appears in the biological material collected from respiratory tract even before clinical symptoms are observed. A positive test result can be expected approximately a week of infection but the results largely depend on the biological material which is under analysis. The virus appears to first infect the individual at the nasopharyngeal tract and it remains there for relatively long — for up to 5–6 weeks therefore viral RNA is first accessible at this site. Approximately at the time virus RNA appears in the respiratory tract but disappears after 2–3 weeks. In sputum and BAL virus RNA appears slightly later, at a time when first clinical symptoms manifest (sometimes earlier), and remains there longer than in biological material collected from other regions of the respiratory tract. Virus RNA is also detectable in feces/stool, where it may persist for a very long time, in the sperm of infected men, and occasionally in blood [1, 2, 5]. From the diagnostic point of view however, biological material other than that collected from respiratory tract is of little importance.

The method of SARS-CoV-2 RNA detection recommended by Polish and international guidelines is reverse transcription real-time polymerase chain reaction. It should be kept in mind however that available on the market are also tests based on other technologies of genetic material amplification, eg. transcription-mediated amplification which are comparable to tests based on the PCR method in terms of sensitivity and efficiency (test cases per hour) [6–8].

RNA test cycle lasts 3–4 hours for most of the current tests and involves: nucleic acids extraction, amplification of genomic fragments and internal control [1, 3, 5, 8]. Every clinician dreams of easy access to rapid diagnostic molecular tests for covid-19. On the market there are tests that provide results in less than 1.5 hours. So far however, relatively little is known about their sensitivity and specificity [9, 10].

At present, the DNA sequencing technology, including next generation sequencing (NGS) has a merely cognitive value due to high costs, access to high-tech equipment and qualified personnel [10, 11].

When SARS-CoV-2 infection is suspected, the key issue is to decide where molecular testing is to be performed. The list of laboratories authorized to perform molecular diagnostics of the new coronavirus is available at the website of the Ministry of Health [12]. According to December 2020 data, the list includes 269 such diagnostic laboratories all of which must meet the appropriate biosafety criteria for BSL2 laboratories (employees are required to have appropriate personal protective equipment, tests are subject to quality control, including preliminary assessment of the accuracy of positive and negative results). The highest safety and quality standards are met by laboratories that perform nucleic acids extractions with fully automated methods.

Pursuant to the decree of the Council of Ministers of May 16, 2020, the laboratories contracted by the National Health Fund to perform SARS-CoV-2 RNA testing are obliged to accept electronic orders through the EWP electronic system [13] in which case submission of paper orders is not mandatory. This procedure however may induce pre-analytical errors caused by illegible data on tubes with material samples. To reduce such error risk, it is useful to have a simplified list of samples.

Appropriate collection of analytical material — key stage of the diagnostic process. A comprehensive/in depth description of the appropriate procedure for this stage is presented in the national recommendations developed by the National Consultant in Medical Microbiology (prof. Katarzyna Dzierżanowska-Fangrat) as well as regional consultants in medical microbiology and available on the internet [2]. It is worth noting that the type of material collected from the respiratory tract depends on many factors (symptoms and clinical condition of the patient, access to analytical material). The preferred material for testing is nasopharyngeal swab (simultaneous taken from the throat and nasal mucosa). It is equally important to use proper swabs. Swabs with viral transport medium, although it is also acceptable to use phosphate — buffered saline (PBS). An excellent choice are dacron, rayon and silk swabs. Calcium alginate swabs are not recommended. In consideration of personnel safety, following collection of material from the patient, the swab should be broken at maximum 2/3 of tube-length, placed inside the tube and the tube tightly closed. If the swab is too long and in contact with the stopper, it may straighten abruptly while the tube is being opened and the biological material may spill out exposing the laboratory technician/diagnostician to infectious material.

According to WHO criteria, detection of a fragment of one virus gene is sufficient to confirm the infection in the area with community circulating virus [3]. The Polish criteria are more stringent as they impose detection of fragments of at least two different SARS-CoV-2 genes [2]. In the Depart-

ment of Virology of the Institute of Hematology and Transfusion Medicine (IHTM), COVID 19 diagnostics is performed on the LightCycler 480 Instrument II thermal cycler (Roche Diagnostics) which analizes nucleic acids extracted in the EMAG® automatic apparatus (Fig. 1). Amplification using the SARS-COV-2 R-GENE® test allows for simultaneous checking of the presence of fragments of two viral genes — the gene encoding nucleocapsid (N) protein and the gene encoding the RNA-dependent RNA polymerase (RdRp) protein. For the result to be valid, it is necessary to obtain a positive result of the internal extraction and inhibition control of the sample that is added to the analytical material during nucleic acids extraction. In the absence of amplification of both regions of the virus — N and RdRp, a negative result is obtained while amplification of both regions indicates infection. Either way the diagnostic procedure is completed. When amplification of only one of the two regions occurs, it is necessary to perform another PCR reaction, in which two genes are amplified — a fragment of the region coding for the envelope protein (E gene) and the HPRT1 gene for cell control of the tested material. A positive result for E gene confirms Sarbecovirus infection, a negative result indicates no infection or weak viral load below detection limit (Table 1) [14].

Interpretation of SARS-CoV-2 RNA test results

In the light of scientific data, a single result of the SARS-CoV-2 RNA test does not necessarily determine the status of the individual. A single negative result does not exclude infection and

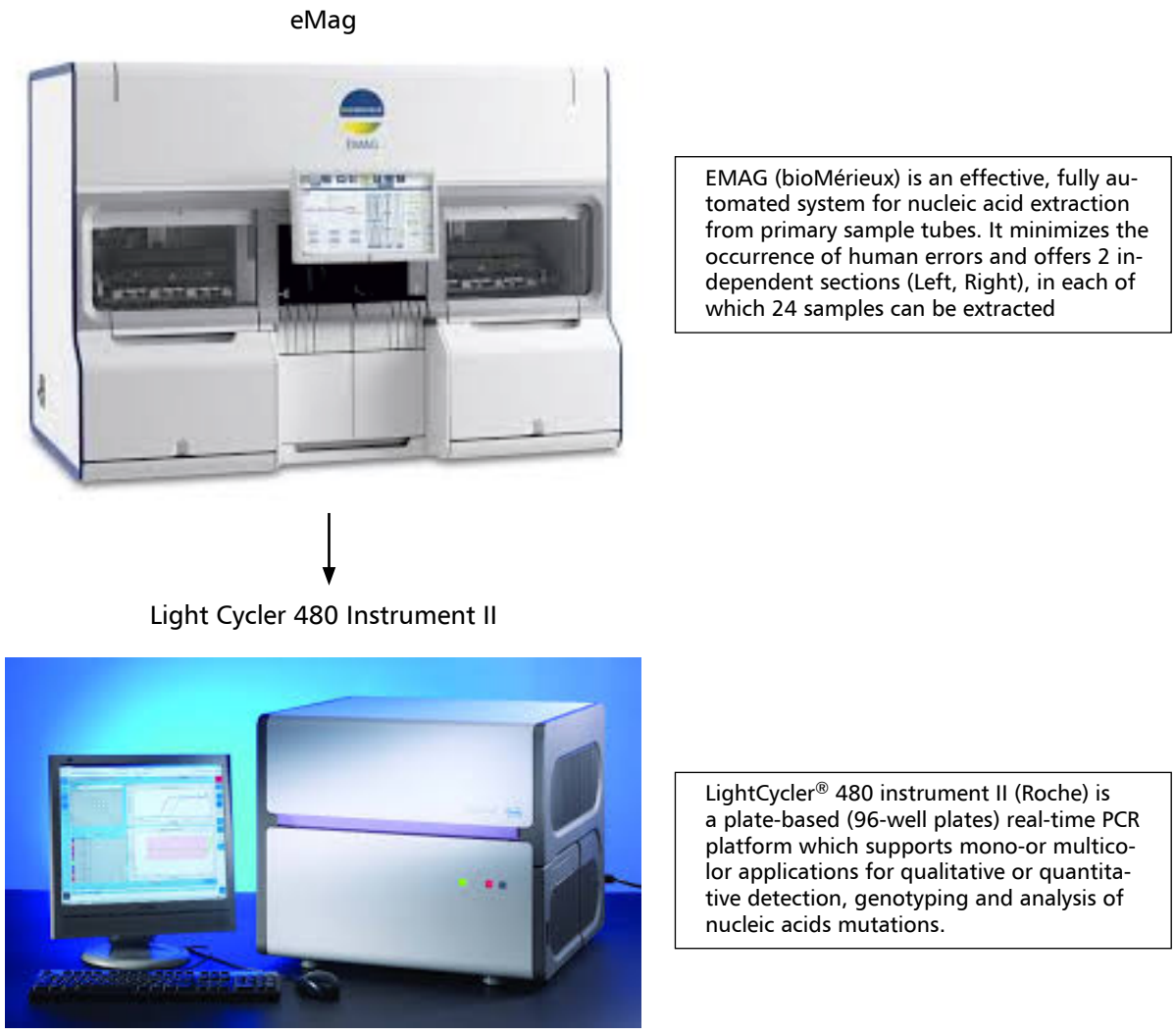

Figure 1. Organization of covid-19 diagnostic test (Department of Virusology IHTM)

Table 1. Interpretation of Multiplex Real-Time PCR results; algorithm for results discrepant in screening test

	Target value of Ct or ΔCt [IC1 sample-IC 1W0]			
N gene (539 nm)	+	+	–	–
IC 1sample — IC 1W0 (560 nm)		≤ 3 Ct or > 3 Ct	≤ 3 Ct	> 3 Ct
RdRp gene (670 nm)	+	–	+	–
	INTERPRETATION			
	SARS-CoV-2 detected	Unequivocal result (recommended test with PCR2 and/or rerun with PCR1)	No SARS-CoV-2 virus detected (or < LoD)	Invalid result (inhibition/weak extraction
PCR2 (Sarbecowirus)				
	Target value Ct or ΔCt [IC1 sample-IC 1W0]			
E gene (530 nm)	+		–	–
IC 1sample — IC 1W0 (560 nm)	≤ 3 Ct or > 3 Ct		≤ 3 Ct	> 3 Ct
Cell cycle control (670 nm)	+ or-	< 35 Ct	≥ 35 Ct lub –	+ or –
	INTERPRETATION			
	Sarbecovirus detected	No Sarbecovirus detected (or < LoD)	No cells detected (news sample testing)	Invalid result (inhibition/weak extraction

should not be treated as the only diagnostic criterion, especially when the clinical picture does suggest COVID-19 infection or the patient was exposed to close, unprotected contact with a confirmed case of COVID-19, regardless of the type and intensity of clinical symptoms. National recommendations clearly state that hospitalized patients with negative result in the first molecular test should be tested again if: a) likelihood of infection based on epidemiological history, clinical picture and chest imaging is high (the test should be ordered within 24–48 hrs. of the first sample collection b) symptoms from the respiratory system worsen (the test should be ordered within 24–48 hrs. of first sample collection and c) when the patient requires intubation and there is a chance of collecting material from the lower respiratory tract.

The indeterminate test result on the other hand, does not clearly point to infection and a repeat test is required after 1–2 days [1, 2, 15].

Tests for SARS-CoV-2 RNA detection

We are currently witnessing unprecedented, spectacularly intensive production of various tests — according to January 21th 2021 records at least 300 different manual detection tests for SARS-Cov-2 are available worldwide and about 150 automated procedures. And the number is expected to grow [16]. The market is flooded with new products for nucleic acids detection at a pace that precludes quality assessment. Literature reports on independent evaluation tests are still scarce [17–19] so each laboratory should perform validation of the tests used or rely on independent evaluation.

While choosing a SARS-CoV-2 RNA detection test the following aspects should be considered. The COVID-19 molecular diagnostic test must be equipped with an internal control to allow assessment of the accuracy of the diagnostic process which includes nucleic acid extraction and amplification of genomic fragments. It is also

advisable to be able to check the procedure of sample collection. Obtaining the final result when the result is positive is markedly accelerated by the simultaneous amplification of at least two regions of the viral genome, and for indeterminate results, it is good to perform a conclusive test (second PCR). The choice of a diagnostic test should be preceded by analysis of the precision of the test results (repeatability and reproducibility) (Ct/Cp value, cycle threshold/crossing point) and analytical sensitivity expressed in cp/ml [copies/ /mL] with a 95% confidence interval. Tests used in Poland should be CE IVD-marked, although this is not always a 100% guarantee of quality. Specificity is yet another key aspect of tests considered for COVID-19 diagnostics. It is not enough to present the percentage value of this parameter, but the manufacturer should present other respiratory pathogens and substances analyzed for cross-reactions. This evaluation step is crucial as the tests are used to amplify fragments of the virus genome from swabs collected from respiratory tract of individuals with respiratory diseases who often take medication that may interfere with the amplification of nucleic acids. The procedure of cross-reaction differentiates between SARS-CoV-2 and infections with other pathogens (viruses, bacteria, fungi).

A parameter which is mandatory in all manufacturer manuals is test sensitivity. Manufacturers often provide values approximating 100%, which seems hardly reliable unless the reference method is specified. It is known for a fact that the methods used do not detect all SARS-CoV-2 infections, and clinical sensitivity depends on the stage of disease.

Table 2 presents six molecular-based diagnostics tests for SARS-CoV-2, three of them were used at the Department of Virology (IHTM) since pandemic outbreak up to June 2020. More widely used were at least: *genesig® kit* for the detection of coronavirus (COVID-19) in real-time PCR (Primerdesign Ltd) and *Bosphore® Novel Coronavirus (2019-nCoV) Detection Kit v2* (Anatolia geneworks) they were distributed by the Central Reserve Base for laboratories on the COVID-19 List.

Table 2 also includes information on the first Polish RNA detection tests for the new coronavirus as well as a test for CoBAS equipment used in Polish blood transfusion service for massive testing.

Attention should also be paid to the ongoing research on increasing the throughput of the diagnostic procedures; by specimen pooling [20, 21] and simplification of the nucleic acid extraction step [22].

Table 2. Comparison of tests for SARS-CoV-2 RNA research

Test	Validated nucleic acid extraction system	Amplified genomic fragments	Internal control	Control of collection	Analytical sensivity (95% LOD)	Clinical sensivity	Specificity	The range of assessed cross-reactions	No. of tests per 8 hrs
Genesig® kit for (COVID-19) detection in real-time PCR (CE IVD) Primer design Ltd	RNA Extraction Systems (CE IVD)	SARS-CoV-2 (no distinguished genes)	Yes	No	0,58 copies/ /µl	98%	100%	Influenza A: H1N1, H3N2; Influenza B: Victoria, Yamagata; RSV A, RSV B, Coronavirus: NL63, 229E, HKU, OC43	138 (EMAG®)
Bosphore® Novel Coronavirus (2019-nCoV) Detection Kit v2 Anatolia geneworks	Magnesia® 16 Nucleic Acid Extraction System — Magnesia® Viral Extraction Kit (Anatolia Geneworks)/ Magrev® 24 Stand — Magrev® Viral DNA/RNA Extraction Kit (Anatolia Geneworks)/Bosphore® Viral RNA Extraction Spin Kit (Anatolia Geneworks)	Orf1ab (PCR1), E (PCR2)	Yes	No	25 copies/ /rxn	No data	No data	Enterowirus, Parainfluenza-1, Parainfluenza-2, Parainfluenza-4, RSV-A, Rhinovirus, Bocavirus, Parechovirus, Influenza A, Influenza B, Coronavirus 229E, Coronavirus OC43, Coronavirus HKU1, Coronavirus NL63 and SARS	138 (EMAG®)
ARGENE® SARS--COV-2 R-GENE® BioMerieux	EMAG®, NUCLISENS® easyMAG®,MagNA Pure Compact, MagNA Pure 96, QIAsymphony SP	N, RdRp (PCR1), E (PCR2)	Yes	Yes (HPRT1)	380 copies/ /ml	100% — SARS--CoV-2 (PCR1 and PCR2) 98,7% — Sarbecovirus	100%	**PCR1:** viruses: human coronavirus: 229E, NL63, OC43, HKU1, coronavirus SARS i MERS, adenovirus, human metapneumovirus (hMPV), parable virus 1–4, flu A, flu B, enterovirus, human respiratory syncytial virus (RSV), parechovirus i rinovirus; bacteria: *Chlamydia pneumoniae, Haemophilus influenzae, Legionella pneumophila, Streptococcus pneumoniae, Streptococcus pyogenes, Bordetella pertussis, Mycoplasma pneumoniae* **PCR2:** viruses: human coronavirus: 229E, NL63, OC43, HKU1, coronavirus MERS, adenovirus, human metapneumovirus (hMPV), parable virus 1–4, flu A, flu B, enterovirus, l human respiratory syncytial virus(RSV), parechovirus i rinovirus; bacteria: *Chlamydia pneumoniae, Haemophilus influenzae, Legionella pneumophila, Streptococcus pneumoniae, Streptococcus pyogenes, Bordetella pertussis, Mycoplasma pneumoniae*	138 (EMAG®)

Table 2 cont. Comparison of tests for SARS-CoV-2 RNA research

Test	Validated nucleic acid extraction system	Amplified genomic fragments	Internal control	Control of collection	Analytical sensivity (95% LOD)	Clinical sensivity	Specificity	The range of assessed cross-reactions	No. of tests per 8 hrs
Cobas® SARS--CoV-2 Roche Diagnostics GmbH	The cobas® 6800/8800 Systems (extraction/amplification)	Orf1ab, E	Yes	No	0.007 TCID50/ /mL (SARS--CoV-2) (95% CI: 0.005–0.036), 0.004 TCID50/mL (pan-Sarbecovirus) (95% CI: 0.002–0.009)	100% (95% CI: 92.9–100%)	100% (95% CI: 96.3–100%)	Coronavirus: 229E, OC43, HKU1, NL63, MERS coronavirus, SARS coronavirus Adenovirus B (Type 34) Human Metapneumovirus (hMPV) Parainfluenza virus Type 1 Parainfluenza virus Type 2 Parainfluenza virus Type 3 Parainfluenza virus Type 4 Influenza A (H1N1) Influenza B Enterovirus E (Type 1) Respiratory syncytial virus Rhinovirus *Chlamydia pneumonia* *Haemophilus influenzae* *Legionella pneumophila* *Mycobacterium tuberculosis* *Streptococcus pneumonia* *Streptococcus pyrogenes* *Bordetella pertussis* *Mycoplasma pneumoniae* pooled human nasal fluid	About 400
MediPAN-2G+ COVID test Medicofarma	No data	Orf1ab, S	Yes	No	–	> 95%	> 99%	Coronavirus: 229E, OC43, HKU1, NL63, MERS coronavirus, SARS coronavirus, Adenovirus 1, Enterovirus D, Enterovirus E, Influenza A (H1N1), Influenza B, Human metapneumovirus, Rhinovirus B, Respirovirus 1, Respirovirus 3, Rubulavirus 2, Rubulavirus 4, Human orthopneumovirus, *Bordetella pertussis, Condida albicans, Corynebacterium diphtheriae, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Moraxella catarrhalis, Neisseria meningitidis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus salivarius, Streptococcus pneumoniae, Streptococcus pyogenes*	138 (EMAG®)

Table 2 cont. Comparison of tests for SARS-CoV-2 RNA research

Test	Validated nucleic acid extraction system	Amplified genomic fragments	Internal control	Control of collection	Analytical sensivity (95% LOD)	Clinical sensivity	Specificity	The range of assessed cross-reactions	No. of tests per 8 hrs
MediPAN-2G+ FAST COVID test Medicofarma	No data	Orf1ab, S	Yes	No	200 copies mL	> 99%	> 99%	Coronavirus: 229E, OC43, HKU1, NL63, MERS coronavirus, SARS coronavirus, Adenovirus 1, Enterovirus D, Enterovirus E, Influenza A (H1N1), Influenza B, Human metapneumovirus, Rhinovirus B, Respirovirus 1, Respirovirus 3, Rubulavirus 2, Rubulavirus 4, *Human orthopneumovirus, Bordetella pertussis, Condida albicans, Corynebacterium diphtheriae, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Moraxella catarrhalis, Neisseria meningitidis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus salivarius, Streptococcus pneumoniae, Streptococcus pyogenes*	138 (EMAG®)
MediPAN-2G COVID test Medicofarma	No data	Orf1ab, S	Yes	Yes	–	> 95%	> 99%	Coronavirus: 229E, OC43, HKU1, NL63, MERS coronavirus, SARS coronavirus, Adenovirus 1, Enterovirus D, Enterovirus E, Influenza A (H1N1), Influenza B, Human metapneumovirus, Rhinovirus B, Respirovirus 1, Respirovirus 3, Rubulavirus 2, Rubulavirus 4, *Human orthopneumovirus, Bordetella pertussis, Condida albicans, Corynebacterium diphtheriae, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Moraxella catarrhalis, Neisseria meningitidis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus salivarius, Streptococcus pneumoniae, Streptococcus pyogenes*	138 (EMAG®)

References

1. Zalecenia w COVID-19, wersja 1.0 – 23.04.2020, AOTMiT.
2. „Zasady pobierania i transportu materiału do badań metodami molekularnymi rRT-PCR w kierunku SARS-CoV-2," opracowane przez Konsultanta krajowego w dziedzinie mikrobiologii lekarskiej – Katarzynę Dzierżanowską-Fangrat oraz konsultantów wojewódzkich w dziedzinie mikrobiologii lekarskiej.
3. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Interim guidance. 19 March 2020. https://www.who.int/publications/i/item/10665-331501.
4. https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-at-higher-risk.html.
5. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA. 2020; 323(22): 2249–2251, doi: 10.1001/jama.2020.8259, indexed in Pubmed: 32374370.
6. Gorzalski AJ, Tian H, Laverdure C, et al. High-throughput transcription-mediated amplification on the hologic panther is a highly sensitive method of detection for SARS-CoV-2. J Clin Virol. 2020; 129: 104501, doi: 10.1016/j.jcv.2020.104501, indexed in Pubmed: 32619959.
7. Smith E, Zhen W, Manji R, et al. Analytical and clinical comparison of three nucleic acid amplification tests for SARS-CoV-2 detection. J Clin Microbiol. 2020; 58(9), doi: 10.1128/JCM.01134-20, indexed in Pubmed: 32571894.
8. Pham J, Meyer S, Nguyen C, et al. Performance characteristics of a high-throughput automated transcription-mediated amplification test for SARS-CoV-2 detection. J Clin Microbiol. 2020; 58(10), doi: 10.1128/JCM.01669-20, indexed in Pubmed: 32727828.
9. Broughton JP, Deng X, Yu G, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020; 38(7): 870–874, doi: 10.1038/s41587-020-0513-4, indexed in Pubmed: 32300245.
10. Michel D, Danzer KM, Groß R, et al. Rapid, convenient and efficient kit-independent detection of SARS-CoV-2 RNA. J Virol Methods. 2020; 286: 113965, doi: 10.1016/j.jviromet.2020.113965, indexed in Pubmed: 32891677.
11. Chiara M, D'Erchia AM, Gissi C, et al. Next generation sequencing of SARS-CoV-2 genomes: challenges, applications and opportunities. Brief Bioinform. 2020 [Epub ahead of print], doi: 10.1093/bib/bbaa297, indexed in Pubmed: 33279989.
12. Wpis do wykazu laboratoriów COVID-19 Ministerstwa Zdrowia: laboratoriów (stan na 22 maja 2020 r.) /https://www.gov.pl/web/zdrowie/lista-laboratoriow-covid/.
13. Rozporządzenie Rady Ministrów z dnia 16 maja 2020 r. w sprawie ustanowienia określonych ograniczeń, nakazów i zakazów w związku z wystąpieniem stanu epidemii Dziennik Ustaw 2020 r. poz.: 878.
14. Instrukcja obsługi ARGENE®SARS-COV-2 R-GENE®-[BIOMÉRIEUX Ref 423720, 055836 — 01 — 2020-04].
15. https://gis.gov.pl/aktualnosci/definicja-przypadku-na-potrzeby-nadzoru-nad-zakazeniami-ludzi-nowym-koronawirusem-sars-cov-2/.
16. https://www.finddx.org/covid-19/pipeline/.
17. Abdalhamid B, Bilder CR, McCutchen EL, et al. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. Am J Clin Pathol. 2020; 153(6): 715–718, doi: 10.1093/ajcp/aqaa064, indexed in Pubmed: 32304208.
18. Zhen W, Manji R, Smith E, et al. Comparison of four molecular diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens. J Clin Microbiol. 2020; 58(8), doi: 10.1128/JCM.00743-20, indexed in Pubmed: 32341143.
19. Ishige T, Murata S, Taniguchi T, et al. Highly sensitive detection of SARS-CoV-2 RNA by multiplex rRT-PCR for molecular diagnosis of COVID-19 by clinical laboratories. Clin Chim Acta. 2020; 507: 139–142, doi: 10.1016/j.cca.2020.04.023, indexed in Pubmed: 32335089.
20. Abdalhamid B, Bilder CR, McCutchen EL, et al. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. Am J Clin Pathol. 2020; 153(6): 715–718, doi: 10.1093/ajcp/aqaa064, indexed in Pubmed: 32304208.
21. Wacharapluesadee S, Kaewpom T, Ampoot W, et al. Evaluating the efficiency of specimen pooling for PCR-based detection of COVID-19. J Med Virol. 2020; 92(10): 2193–2199, doi: 10.1002/jmv.26005, indexed in Pubmed: 32401343.
22. Fomsgaard AS, Rosenstierne MW. An alternative workflow for molecular detection of SARS-CoV-2 — escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020. Euro Surveill. 2020; 25(14), doi: 10.2807/1560-7917.ES.2020.25.14.2000398, indexed in Pubmed: 32290902.