ÖGLMKC

Österreichische Gesellschaft für
Laboratoriumsmedizin und Klinische Chemie

Version 1.6, 07.11.2020

Significant changes compared to the previous version:

- Update 1.2. Direct virus detection using an antigen test
- New chapter 1.2.1. Evaluation of the results of antigen tests

Laboratory diagnostics for coronavirus SARS-CoV-2

Due to the increasing number of COVID-19 cases in Austria, which are caused by the coronavirus SARS-CoV-2, the Austrian Society for Laboratory Medicine and Clinical Chemistry (ÖGLMKC) would like to summarize the cornerstones of laboratory diagnostics for COVID-19. The recommendations are also coordinated with the Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP). This summary corresponds to the current state of knowledge; new scientific knowledge of COVID-19 is currently being published according to sometimes greatly shortened peer review procedures and requires ongoing updating and a rational evaluation.

1. Diagnosis of a SARS-CoV-2 infection

1.1. Direct virus detection using PCR

The laboratory diagnostic gold standard for the diagnosis of an infection with coronavirus SARS-CoV-2 is the direct virus detection from respiratory secretions by means of polymerase chain reaction (PCR) or other nucleic acid amplification techniques (NAT).

1.1.1. Sampling and transportation

Samples are obtained from the upper respiratory tract using an oro or nasopharynx smear. It is particularly important to rinse the throat (with water) before taking an oropgharyngeal smear or to clear the nose by blowing the nose before taking a nasopharyngeal smear. This means that PCR inhibitors can be removed or reduced. Alternatively, samples from the deep respiratory tract (induced sputum, tracheal secretion or bronchoalveolar lavage) can also be used in the appropriate clinical situation. Coronavirus SARS-CoV-2 has also been detected by means of

PCR in stool and in individual cases in urine, blood/plasma and liquor, but these materials are currently not recommended for the primary diagnosis of COVID-19 disease.

Samples must be taken by appropriately trained personnel. The self-acceptance of samples is assessed critically by the ÖGLMKC. On the one hand, the quality of the sampling must be guaranteed to avoid false negative results. Alternative acceptance methods must therefore be fully validated according to medical standards. On the other hand, a clear identification of the patient is necessary, particularly if official decisions are to be derived from the test result.

Coronaviruses are enveloped RNA viruses, the virus particles and their single-stranded RNA genome are therefore sensitive to surfactants and RNAses. For this reason, special requirements for sampling, storage and transport must be made in the preanalytics in order to avoid false-negative results. In the case of swabs, it must be ensured that suitable swabs and transport media are used for virus detection ("virus swabs" with appropriate transport medium or, if necessary, dry sterile swabs with a small amount (1 - 3 ml) of sterile NaCl solution suitable for molecular genetic analyzes, at least none Gel swab). The selection of a suitable swab should be checked in the course of establishing the test.

After collection, the samples should be brought to the laboratory as soon as possible. If samples need to be stored, this can be done at 2-8 ° C for a maximum of 3 days or according to the manufacturer's instructions. Samples must be sent as "biological substance, category B" of the UN no. 3373 according to the specifications of packing instruction P650. For longer transport times, the shipment should be refrigerated.

1.1.2. Sample handling in the laboratory for virus detection

The handling of respiratory samples in the context of laboratory diagnostics of SARS-CoV-2 infections falls into the category "non-targeted activities" and should be restricted to specially trained laboratory personnel. Updated German-language guidelines for obtaining and handling potentially contagious sample material can be found on the websites of AGES (Austrian Agency for Health and Food Safety), the RKI (Robert Koch Institute) and ABAS (Committee for Biological Agents). In particular, we allow ourselves to refer to the recommendations for handling test material from Covid 19 positive / suspect patients in the laboratory of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP). Link: https://www.oeghmp.at/media/empfipps_zum_umgang_mit_untersuchungsmaterial_von_covid-19-positiven-verdaechtigen_patienten_im_labor.pdf

In a nutshell, non-targeted activities, such as sample preparation and preparation or inactivation for molecular biological tests (PCR) are carried out under the conditions of biological safety level 2 (BSL-2). All activities that can lead to the release of droplets or aerosols with SARS-CoV-2, e.g. the opening of sample vessels with respiratory material must be carried out in a class 2 safety workbench. In addition to the general safety precautions such as protective gowns and gloves, breathing protection measures (at least FFP-2; filtering face piece, fine dust mask) are recommended to wear safety glasses.

Targeted activities with SARS-CoV-2 (virus isolation, neutralization test or similar) may only be carried out by specially trained personnel in security level 3 (BSL-3) facilities.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

1.1.3. PCR test

1.1.3.1. General

A number of commercial test systems from various suppliers are available for the PCR detection of SARS-CoV-2. Typically, viral nucleic acid (RNA) is extracted from the sample first. From this, after reverse transcription (RT), a PCR (or another NAT such as isothermal amplification) is carried out to detect virus-specific nucleic acids.
Depending on the test system, the analysis steps are automated to different degrees. Roughly summarized, different categories of test systems can be distinguished:

- **Manual or semi-automatic RNA isolation and RT-PCR:** Processing of several samples simultaneously (in batch), medium sample throughput, high molecular genetic expertise of the laboratory staff required.
- **Fully automated SARS-CoV-2 PCR**: Fully automated SARS-CoV-2 PCR: Fully automated extraction / amplification systems for the detection of SARS-CoV-2. These devices can process many samples (e.g. 94 samples) at the same time and thus allow a high sample throughput.
- **Point-of-care-systems:** Cartridge systems for the SARS-CoV-2 PCR. These complete systems are designed for ease of use and allow fully automated virus PCR in a point-of-care setting. For the individual sample, the time to the analysis result is short (less than an hour), which is why these systems are also referred to as (molecular) rapid tests. In addition to the analysis time of the individual sample, the sample throughput of the respective system must also be taken into account. Such systems are particularly suitable for rapid analysis of individual samples in a point-of-care setting.

The availability of reagents for PCR tests and RNA extraction as well as smear systems has improved significantly. Both medical laboratories and manufacturers of commercial test systems continue to work on increasing the analysis capacities. The ÖGLMKC explicitly endorses and supports collaborations to further increase the analysis capacity, but points to the absolute necessity of uncompromising quality control of the SARS-CoV-2 PCR analysis in order to guarantee valid and reproducible test results for all patients.

Successful participation in round-robin tests at regular intervals is an essential prerequisite for qualitative analysis. In Austria, the Austrian Society for Quality Assurance and Standardization of Medical Diagnostic Examinations (ÖQUASTA) offers round-robin tests for SARS-CoV-2 diagnostics.

1.1.3.2. Molecular genetic basis

Established Established in-house and commercial test systems are mostly based on the detection of 2 gene sequences (targets) of SARS-CoV-2. One gene sequence is usually selective for the genus Betacoronavirus and one gene sequence is specific for the Cladus SARS-CoV-2.

The test systems used to date usually detect 2 sequences of the following genes: N (nucleocapsid), E (envelope), S (spike) and RdRP (RNA-dependent RNA polymerase). According to the updated WHO Guidelines for Laboratory Analysis (03/20), if the spread of SARS-CoV-2 in a region is advanced, a simplified workflow with amplification of only one specific region (e.g. PCR detection of the E gene) can be used (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

guidance/laboratory-guidance). In addition, an additional internal control that checks the nucleic acid extraction and amplification as well as the integrity of the reagents must ensure the validity of the test results.

The described detection limit of the first PCR assays for SARS-CoV-2 is around 10 copies of viral nucleic acids per reaction. (J Clin Microbiol. 2020 Mar 4. pii: JCM.00310-20. Doi: 10.1128 /JCM.00310-20.; Euro Surveill. 2020 Mar; 25 (9). Doi: 10.2807/1560-7917.ES.2020.25.9.2000173). Further studies have shown that the sensitivity of assays for the detection of SARS-CoV-2 RNA differs and can differ by more than 1 x log10 (Matheeussen V et al., Eurosurveill., Jul 9; Görzer et al., J Clin Virol 2020: 129, 104537). The detection limit depends on the target sequence used, but also on other factors. The manufacturer's information must be taken into account for the respective assay and verified by comparative measurements in the laboratory before the test is introduced. The ÖGLMKC recommends the use of an assay with a sufficiently low detection limit and a corresponding review of the entire test performance as part of external quality assurance (including samples with low viral load in round-robin test programs).

The ÖGLMKC expressly recommends (if available) the use of CE/IVD-certified assays for the detection of SARS-CoV-2 RNA. If in individual cases it is necessary to introduce an in-house PCR test for the detection of SARS-CoV-2 RNA because CE-marked tests are not available, the test performance must be thoroughly validated and documented by the performing laboratory.

1.1.4. Assessment of PCR Results

The duration of detection of viral RNA in the nasopharynx secretion seems to be subject to large individual fluctuations and is 12 days (1-24 days) in the median, according to a series of cases. In > 80% of patients, the detection is positive for at least 7 days (JAMA. 2020 Mar 3. doi: 10.1001/jama.2020.3204). In some cases, a PCR positivity for >25 days was also described. A recent work describes a detailed time history of the viral load in different sample materials. At the beginning of the symptom, the concentration of viral RNA was high, decreased rapidly in throat swabs during the course of the disease and was typically detectable there for about two weeks, while in samples of the deep respiratory tract (induced sputum) and in the stool an extended viral excretion was observed (Nature 2020 April 1. doi: 10.1038/s41586-020-2196-x (2020)).

Of great practical relevance is that in some confirmed COVID-19 cases the virus RNA is only intermittently detectable (JAMA. 2020 Mar 3. doi: 10.1001/jama.2020.3204). This phenomenon may have pre-analytical (sample handling, experimental use of antivirals) and analytical reasons. Typically, however, it is necessary to be monitored in the late course of the disease, when the viral load in the nasopharynx secretion is low and is at the detection limit of the PCR method. When the load of the virus is low, correct sample collection is also particularly important for virus detection. Therefore, to document that virus excretion is no longer carried out, a twice-negative PCR test is carried out at intervals of ≥ recommended for 24 hours. (https://www.ecdc.europa.eu/en/publications-data/novel-coronavirus-sars-cov-2-discharge-criteria-confirmed-covid-19-cases)

1.1.4.1. Positivity for only one PCR Target

If a PCR test is based on the amplification of two (or more) target sequences of SARS-CoV-2 and only one is positive while the other is negative, there are several causes to consider:

- The amount of virus in the sample is at the detection limit of the test

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

- A technical error in the analysis of the sample (both false positive and false negative possible)
- A mutation in one of the target sequences of SARS-CoV-2
- The presence of a coronavirus other than SARS-CoV-2

In these cases, the PCR result shall be evaluated in combination with the raw data (early or late amplification of the target) and the molecular genetic characteristics of the assay. If a technical error can be ruled out, in the current epidemiological situation it is recommended to consider the amplification of only one PCR target as a positive test result and thus as an indication of the presence of an infection with SARS-CoV-2. This is due to the consideration that only one human pathogenic beta coronavirus is currently circulating, that SARS-CoV-2 has a genetic diversity, that "weak-positive" or "non-evaluable" results may lead to uncertainties in reporting and regulatory procedures, and that any false-negative results could undermine adherence to isolation and quarantine measures.

To differentiate from this is that some test systems are primarily aimed at the detection of gene sequences of the genus betacoronavirus or the subgenus sarbecovirus, so that the specific detection of SARS-CoV-2 would require subsequent sequencing of the PCR product (https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf?sfvrsn=af1aac73_4) https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.

If only one gene sequence can be detected repeatedly in a patient and the result is not at the detection limit of the test, further confirmation tests should be considered. Where appropriate, the specifics should be forwarded to a reference laboratory for SARS-CoV-2 after prior contact; the Austrian Reference Laboratory is the Center for Virology of the Medical University of Vienna.

1.1.4.2. Negative PCR Results

A one-time negative PCR result does not 100% rule out SARS-CoV-2 infection. If there are reasonable grounds for suspicion of SARS-CoV-2 infection and initial negative PCR outcome, a re-sampling and examination should be agreed between the clinician and the laboratory physician. This recommendation is supported by series of cases according to which PCR may initially be negative in epidemiologically, clinically and CT morphologically defined suspected cases (Lancet. 2020 Feb 15;395(10223):514-523. doi: 10.1016/S0140-6736(20)30154-9. Radiology. 2020 Feb 12:200343. doi: 10.1148/radiol.202020343.).

False-negative results may be based in particular on pre-analysis and are based, for example, on poor sample quality, long sample storage, improper sample transport or unfavorable timing of sampling, based on the course of the disease. Inhibition of the PCR or mutation of the virus may play a role in analytics (Clin Infect Dis. 2020 Mar 4. pii: ciaa203. doi: 10.1093/cid/ciaa203.). The former can be detected by using an internal control for nucleic acid extraction and amplification.

1.1.4.3. Consideration of the Ct-value

In the literature, a correlation between the viral load in the test material and the cultivability of the viruses contained in the sample in cell culture is described (Eur J Clin Microbiol Infect Dis 39, 1059-1061 (2020). Doi: 10.1007 / s10096-020-03913-9). The PCR positivity at a later point in time in the course of the reaction (usually indicated as a high Ct value) corresponds approximately to a low viral load in the

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

sample. The recommendation of the Ministry of Health for the "release of COVID-19 cases from isolation" of July 9th, 2020, based on the recommendations of the RKI, names a Ct value of greater than 30 as the limit for a weakly positive sample.

From a laboratory diagnostic point of view, a generally valid definition of such a limit value should be questioned. Most SARS-CoV-2 PCR tests are not validated for quantification of the viral load. The Ct value of a sample is decisively influenced by the pre-analysis (type of sample collection, sample storage), the RNA extraction and the PCR method. Proficiency testing therefore shows relevant differences in the Ct value of the same sample in different laboratories (Görzer et al. Journal of Virology. Doi: 10.1016 / j.jcv.2020.104537 and Matheeussen et al. Euro Surveill. Doi: 10.2807 / 1560-7917.ES. 2020.25.27.2001223). However, a good match between the Ct values of a particular test and those of the published tests is a basic requirement for this assessment. This requires specific internal and external quality controls and should therefore also be considered separately in future round robin programs.

Regardless of analytical considerations, it is essential to take into account that the relationship between the viral load (or Ct value) and the ability of the viruses to be grown in cell culture, especially in samples in the late course of the disease of COVID-19 patients (sometimes several weeks after the onset of symptoms), is ascertained has been. However, there are insufficient data on the relationship between viral load at the beginning of the disease and infectivity. Correspondingly, the considerations of the RKI regarding the consideration of the Ct value relate primarily to the discharge management of patients after the end of the symptomatic phase of illness.

When interpreting the Ct value, it is therefore essential to take into account several influencing factors such as the point in time in the course of the disease and the quality of the sampling as well as the type of material or the smear location, the processing and the test system used. From the point of view of the ÖGLMKC, no reliable statement on infectivity can be derived from the Ct value alone, without taking these influencing factors into account. In this context, the development of quantitative SARS-CoV-2 PCR tests, which better correspond to the desire for a quantitative assessment of the viral load, would be required.

1.1.5. Pool testing

In so-called pool testing, a defined amount of the starting material from several individual samples is combined to form a pool and then only the resulting pool sample is analyzed using PCR. If the result is negative, it is assumed that every sample contained in the pool is to be regarded as negative. If the pool is tested positive, the pool is dissolved and the samples contained are analyzed individually. The pool testing method is primarily aimed at increasing efficiency, mostly with limited test resources (Hanel et al, arXiv: 2003.09944). To minimize the risks of a potential sample mix-up, the sample pooling itself should also be largely automated, if possible.

From a laboratory point of view, it must in any case be checked whether the pool testing has been checked as part of the test approval (CE / IVD) and whether the test is approved for this application. If this is not the case, the responsibility lies with the laboratory carrying out the work and a documented validation must be carried out in the laboratory. Since the increasing dilution effect with increasing pool

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

size leads to a loss of sensitivity and therefore samples with low virus concentrations may no longer be detected, it is recommended that the method only be used for non-event-related or epidemiological investigations, such as pool testing of COVID-19 Suspected cases are not advised. The maximum pool size is limited both by the detection limit of the test procedure and by the epidemiological situation. From an epidemiological point of view, in conjunction with the currently available literature (J Med Virol. Doi: 10.1002 / jmv.25971), it is recommended not to exceed a pool size of a maximum of 10 samples per pool, depending on the underlying prevalence. If the pool size is too high, if the prevalence is high, too many pools have to be closed and the method is no longer efficient or resource-saving. However, depending on the analytical sensitivity of the test procedure, the maximum acceptable pool size can also be significantly there are less than 10 samples.

1.2. Direct virus detection by antigen test

The antigen test is a direct virus detection which immunologically detects viral proteins in respiratory sample materials. **Point-of-care** systems or quick test formats are mainly used for this. In contrast to the PCR test, the antigen test does not require any special laboratory equipment and can be carried out outside of medical laboratories. The rapid availability of the results means that rapid antigen tests are suitable for decentralized testing of symptomatic persons as part of the differential diagnosis of respiratory infections.

The **detection limit** of antigen tests is several orders of magnitude higher than that of PCR, so that a high concentration of viral particles is required in the sample in order to reliably detect a SARS-CoV-2 infection using an antigen test. For example, the WHO has formulated a minimum detection limit equivalent to 10^6 (acceptable) or better 10^4 (desirable) genome copies/ml for antigen tests (target product profiles for priority) for the detection of acute SARS-CoV-2 infection in symptomatic persons diagnostics to support response to the COVID-19 pandemic, WHO, 2020). With its significantly better analytical sensitivity (detection limit in the range from 10^1 to 10^2 genome copies/ml), the PCR is therefore still the reference method for direct virus detection. Due to the clear differences in the detection limit, alternative methods of sampling (such as throat rinsing fluid) or sample processing (such as pool testing) that have been evaluated for PCR tests cannot be transferred to antigen tests. Sampling and preanalytics must therefore be carried out in accordance with the respective manufacturer's instructions; all deviations from this must be validated separately for the respective test.

In terms of **clinical performance data** for the use of antigen tests in situations in which PCR testing is not available or not available quickly enough, the WHO formulates a sensitivity of ≥80% and a specificity of ≥97% as acceptable or a sensitivity in symptomatic patients of ≥90% and a specificity of ≥99% as desirable (Target product profiles for priority diagnostics to support response to the COVID-19 pandemic, WHO, 2020). When assessing the performance data given by the manufacturer, it must be taken into account whether the patient population examined is representative for the planned use of the antigen test; this also applies in particular to use in asymptomatic persons. Only a few manufacturer-independent evaluations of antigen tests are currently published. However, initial publications and our own data indicate significant differences in performance between the antigen tests examined, which emphasizes the need for manufacturer-independent validation.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

Suitable antigen tests can be a **useful addition to the PCR test capacities** where an initial (pre-) decision about the possible presence of a transmission-relevant infection should be made quickly (on site, POCT) in the early phase of the infection (Antigen detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: interim guidance, WHO, 2020; RKI). The evaluation of the results of in vitro diagnostics basically requires expertise and the inclusion of knowledge about the test indication, the quality of the sampling and the consequences of a positive or negative result (RKI). This applies in particular to the use of antigen tests in a point-of-care setting.

1.2.1. Evaluation of the results of antigen tests

A **negative result** of an antigen test does not rule out an infection with SARS-CoV-2. In particular, if there is a low virus concentration in the sample, as is typical in the early incubation phase and in the late phase of infection, false negative results often occur, so that the clinical sensitivity of the antigen test is inferior to that of the PCR. This must be taken into account when defining areas of application and interpreting negative results. If there is a high clinical suspicion of the presence of a SARS-CoV-2 infection, a new test using PCR is indicated if the antigen test result is negative. If an antigen test is used as part of a regular test concept, this limitation can potentially be partially compensated for by a high frequency of testing.

A **positive result** of an antigen test speaks - with the corresponding symptoms - for the presence of an infection with SARS-CoV-2. False positive results were described to a variable extent (https://www.fda.gov/medical-devices/letters-health-care-providers/potential-false-positive-results-antigen-tests-rapid-detection-sars-cov-2-letter-clinical-laboratory; https://www.medrxiv.org/content/10.1101/2020.11.12.20230292v1). False positive test results can be excluded by confirmation using PCR. Whether this is necessary in individual cases or whether the specificity of the antigen test used is sufficient depends crucially on the probability of the pre-test, so that the current epidemiological situation and the symptoms of the person tested should be taken into account when making this decision.

1.3. Indirect virus detection by antibody test

Immunological tests for serological examination detect antibodies against coronavirus SARS-CoV-2 in the blood of patients which are formed as part of the patient's immune response against SARS-CoV-2. The time course until the detection of antibodies in the context of a SARS-CoV-2 infection can vary individually and has not yet been conclusively investigated for many tests. In general, the start of seroconversion was described about 10-14 days after the onset of symptoms (Nat Med. 2020 Apr 29; doi.org/10.1038/s41591-020-0897-1).

The available antibody tests differ in essential respects:

- **Antibody class:** The test systems either detect IgG, IgM and IgA antibodies against SARS-CoV-2 in isolation or use a sandwich method to bind all reactive antibodies without isotype differentiation (so-called "Total Antibody Assays"). The kinetics of the respective antibody class differ in the course of the disease and therefore, depending on the time of sampling, also has an effect on the clinical sensitivity of an antibody test. Typically, IgM and IgA antibodies appear earlier, while IgG antibodies remain detectable for longer. A comparatively early appearance of IgG antibodies has been described for SARS-CoV-2 (Lancet. 2020 Mar 23; doi.org/10.1016/S1473-3099(20)30196-1.)

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

- **Antibody specificity:** Viral proteins to which the patient's antibodies bind are used for the antibody tests. Different proteins of SARS-CoV-2 can be used as antigen for antibody detection in different test systems. This can make a decisive difference in the specificity and sensitivity of the test. SARS-CoV-2 shows great similarities to SARS-CoV (no longer circulating since 06/2004) and MERS-CoV (origin limited to the Arabian Peninsula with little activity) to the also related, seasonally occurring, low-pathogenic human coronaviruses (HCoV-HKU1, HCoV-NL63, HCoV-OC43 and HCoV-229E) there is a much less similarity. For many antibody tests - according to the current state of knowledge - cross-reactivity with antibodies against seasonal coronaviruses should only play a subordinate role. However, false positive results can be found (to varying degrees) in all antibody tests and are frequently observed after other (viral and non-viral) infections, autoimmune diseases or other conditions with activation of the immune system.
- **Type of test execution:** The types of tests available range from classic laboratory tests such as enzyme-linked immunosorbent assay (ELISA) or technical variants of chemiluminescence immunoassays (CLIA) to "rapid tests" which allow the analysis of individual blood samples without extensive equipment. The latter are often immunochromatographic “lateral flow” methods, which are similar to a pregnancy test strip for home use by laypersons. In contrast, classic laboratory tests are suitable for the standardized processing of large amounts of antibody tests, but they require appropriate equipment and qualified medical personnel.
- **Type of result:** Depending on the type of test system, the result of the antibody test is purely qualitative (positive / negative) or quantitative. Quantitative tests potentially have the advantage of being able to assess the dynamics of the rise or fall in the antibody. There are currently mainly test systems on the market that deliver purely qualitative results.

For many antibody tests on the market, there are currently insufficient data on sensitivity and specificity, which were determined in studies independent of the manufacturer. However, new papers are being published on this subject, mainly so-called “preprints”, ie pre-publications without peer reviews, many of which should appear as reviewed scientific articles in the coming months. It is crucial for the diagnostic application that these data have been collected from a group of patients or healthy persons relevant to the clinical question. For example, data on the clinical sensitivity and specificity of a test, which was collected in COVID-19 patients in the advanced course of the disease, cannot be used for the question of a COVID-19 diagnosis at the onset of symptoms.

If the indication for an antibody test is made, those tests should be used which, according to the current state of the art, have the highest specificity, sensitivity and precision and whose performance data have been verified by the respective laboratory. The ÖGLMKC therefore recommends carrying out serological tests for SARS-CoV-2 only in medical laboratories and using near-patient serological rapid tests only in exceptional cases.

In summary, according to the current state of knowledge, serological tests alone (without PCR) are neither suitable for diagnostic detection nor to rule out an acute infection by SARS-CoV-2. In individual cases, however, it can make sense to carry out anti-SARS-CoV-2 antibody tests in addition to PCR analyzes, especially if several serum samples are examined with a valid antibody test during the course of the disease. Much more important are antibody tests for epidemiological studies to assess the number of unreported cases of COVID-19 infections that have not been confirmed by PCR detection. The ÖGLMKC recommends to refrain from the uncritical use of antibody tests.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

1.3.1. Potential applications of antibody tests against SARS-CoV-2

- According to the current state of knowledge, serological tests are suitable for the detection of antibodies against SARS-CoV-2, especially for **epidemiological analyzes** of infections in the population.
    - A false positive result of an antibody test could lead to the prevalence being overestimated. For this reason, it is necessary to use antibody tests with a very high specificity (usually> 99%) and / or to check positive test results with a second, independent test system.
- In the **diagnosis of a SARS-CoV-2 infection** in an individual patient, the **detection of antibodies is inferior to the direct detection of viruses using PCR**.
    - This applies in particular to the **early phase** of the disease. A false negative result of an antibody test can lead to the fact that the possibility of a SARS-CoV-2 infection in a patient is falsely excluded, especially in the early phase of the disease.
    - Another problem could arise in **immunosuppressed persons** with congenital or acquired disorders of the immune system, which sometimes develop too late, no antibodies or antibodies that are too small for serological detection or that are too small. For this group of people, the informative value of a negative SARS-CoV-2 antibody test is limited.
    - In the **late phase** of the disease, however, a positive antibody test can be diagnostically helpful; especially if there is no meaningful PCR test. The positive force of a positive antibody finding is highest if several blood samples are available at intervals in the course of the disease with a documented seroconversion..
- It is currently assumed that after a SARS-CoV-2 infection there is a certain **immunological protection** against a new infection (the duration of the immunity and the clinical extent are, however, so far unclear). In principle, an antibody detection with a sufficiently specific test is suitable for the detection of a previous infection. At the moment, however, there is still too little conclusive data on which antibodies and in what level reflect effective immunological protection against renewed SARS-CoV-2 infections. The gold standard for the detection of immunologically active antibodies against SARS-CoV-2 is a neutralization test. Whether and how well the antibodies detected with a specific serological test correlate with a virus-neutralizing effect has not yet been adequately proven for most tests.
    - A false positive result of an antibody test can lead to a person incorrectly assuming immunity to SARS-CoV-2. If no protective measures apply to such a person, there is a risk of infection with SARS-CoV-2 and as a result of transmission to contact persons.
    - Serological tests are used in recovered COVID-19 patients (diagnosis confirmed by PCR) to assess the extent of antibody formation. This is of direct relevance, in particular, when plasma preparations are used by recovered persons (in the context of studies) for the passive immunization of sick people. Classic antibody tests can be used here as pre-screening. The gold standard for the detection of immunologically active antibodies is the neutralization test.

1.3.2. Evaluation of a positive antibody result

A positive antibody finding should always be interpreted in the context of the specificity of the test system used, expected seroprevalence and clinical information on the patient. Several SARS-CoV-2 anti-body

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

tests are currently available, which in very large cohorts (> 1000 individuals) have high specificities with> 99% and sometimes. Reach> 99.5%.

- A positive antibody test is most meaningful if the seroconversion is documented in the course of the disease.
- If the clinical presentation and the imaging clearly speak in favor of COVID-19 (i.e. there is a correspondingly high pre-test probability) and the direct virus detection by means of PCR testing was not carried out or was carried out too late (and is therefore negative), a positive antibody result may raise suspicion Confirm COVID-19.
- The positive predictive value of a test result depends primarily on the specificity of the test system used and the prevalence of the disease. As of July 23, 2020, the frequency of COVID-19 cases in Austria is documented with 226 / 100,000 inhabitants or 0.23% of the population. The unreported number of unknown infections is not exactly known, but even assuming a very high number of unreported cases and assuming a multiple of the confirmed cases, the Austria-wide seroprevalence will hardly exceed 1%. In a hypothetical antibody test with a sensitivity of 100% and a specificity of 99%, taking into account the assumed prevalence for the general population of 1%, a positive predictive value of 50% results. In other words, the test is at least as often false positive as true positive even with a high specificity of 99% in the context of a very low prevalence. The value of a positive antibody finding in asymtomatic persons or in patients with atypical clinical presentation must therefore be viewed critically.
- Individual providers have validated their antibody test against a gold standard for the detection of immunologically active antibodies against SARS-CoV-2 (neutralization test). Since SARS-CoV-2 was only discovered relatively recently, it is not yet known how the clinical extent of immunity is to be assessed and how long this lasts. A reduction or loss of neutralizing antibodies within months has been described in some patients. In a statement dated April 24, 2020, the WHO states that there is currently no reliable evidence that people who have recovered from a COVID-19 disease and have developed antibodies are effectively protected from a renewed infection (https://www.who.int/news-room/commentaries/detail/immunity-passports-in-the-context-of-covid-19). The ÖGLMKC therefore recommends that evidence of an immune reaction - regardless of the respective test and specific methodological limitations - <u>not</u> be interpreted as evidence of complete and lasting immunity to SARS-CoV-2 according to the current state of knowledge.

1.3.3. Examples for the interpretation of the results of an antibody test

In the interpretation of the findings of an antibody test, the limitations according to the current data situation should be explicitly mentioned. An example of the interpretation of a positive or negative anti-SARS-CoV-2 IgG test is given below. Without prejudice to this, an individual interpretation of the findings can and should of course also take place. This can also take into account the respective prevalence of COVID-19.

- **Anti-SARS-CoV-2 IgG test positive:**

The positive test result is an indication of an existing or past infection with the SARS-CoV-2 virus. Rare cross-reactivities cannot be ruled out with absolute certainty. Based on the current data, no statement can currently be made on the extent and duration of immunity.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

A positive IgG test does not reliably rule out active infection. In the case of corresponding clinical symptoms or other suspicion of an active infection, a SARS-CoV-2 PCR test from respiratory material is required to clarify the infectiousness.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

- **Anti-SARS-CoV-2 IgG-Test negative:**

A negative test result does not reliably rule out contact with the SARS-CoV-2 virus. The formation of IgG antibodies begins relatively early with SARS-CoV-2 (10-14 days after the onset of symptoms), however, both the time at which the onset and the amount of antibodies formed can vary individually, so that about 3 weeks after the onset of symptoms the majority of COVID-19 sufferers have specific antibodies in their blood. At the same time, more and more cases are reported in the specialist literature in which the antibody formation is very moderate and therefore does not necessarily lead to a positive result in every test system.

In the case of corresponding clinical symptoms or other suspicion of an active infection, a SARS-CoV-2 PCR test from respiratory material is required to clarify the infectiousness and a serological follow-up is recommended.

1.4. Definitions of definitions of the properties of diagnostic tests

1.4.1. Analytical Sensitivity and Specificity

The analytical sensitivity and specificity of a test describe the suitability of a test to detect a particular analyte (e.g. SARS-CoV-2 nucleic acids) in a sample. These characteristics of a laboratory test can be determined by a pure technical validation of the test.

The **detection limit** of a method describes the lowest concentration of the analyte, which can be reliably detected by the test. A test with a low detection limit can detect low concentrations of the analyte and has a **high analytical sensitivity**.

The analytical specificity of a test describes the ability of the test to detect only the desired analyte and not to be influenced by other substances in the sample (in addition to general interference factors, for example, other coronaviruses).

1.4.2. False negative and false positives

Differences between the actual presence of a disease and the result of a laboratory test are called false negative or false positive results.

False negative people are sick patients, which the test mistakenly classifies as healthy.
False positives are actually healthy individuals, which the test mistakenly classifies as sick.
Really negative people are healthy people, which the test correctly classifies as healthy.
Really positive people are sick patients, which the test correctly classifies as sick.

1.4.3. Clinical Sensitivity and Specificity

The clinical sensitivity and specificity of a test describe the suitability of a laboratory test to distinguish the sick and the healthy. These characteristics of a laboratory test can only be collected by clinical validation of the test with patient samples and are only valid for the clinical situation for which the patient group used is representative.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

The **diagnostic sensitivity** describes the proportion of correctly positive test results in patients with a disease and is usually expressed in %. A test has a high diagnostic sensitivity when **few false negative** results occur.

The **diagnostic specificity** describes the proportion of correctly negative test results in healthy people and is usually expressed in %. A test has a high diagnostic specificity when **few false positives** results occur.

1.4.4. Positive and negative predictive value (prediction value)

In addition to its diagnostic sensitivity and specificity, the predictive value of a laboratory test also depends on the frequency or prevalence of the disease in the population. As a result, the predictive value of the same laboratory test changes when the frequency of a disease such as COVID-19 increases significantly in the population.

The **positive predictive value** is the probability of a disease if the laboratory test is positive (pathological). The higher the clinical specificity of a laboratory test and the more frequent a disease occurs, the higher the positive predictive value of the test.

The **negative predictive value** is the probability that a disease can be ruled out if the laboratory test is negative (normal). The higher the clinical sensitivity of a laboratory test and the less frequently a disease occurs, the higher the negative predictive value of the test.

1.5. Quality assurance

1.5.1. Legal basis

The testing of sample material for human diagnostic purposes with test kits and devices is subject to the Austrian Medical Devices Act (MPG) and its aim is to ensure the safety and high-quality care of patients and society with medical devices and laboratory diagnostic tests. Compliant, high-quality and therefore legally permissible testing therefore requires

- CE marked test kits or
- test kits correctly validated in-house by the laboratory or
- by means of an exemption from the Ministry of Health in accordance with section 32 (1) at the request of the manufacturer or test kits approved on the basis of section §113a

The requirements of the MPG, including quality assurance, apply to every laboratory that carries out tests for patients, regardless of the legal status or any crisis situations, and are not overridden by the Epidemic Act or related regulations. The Austrian Medical Devices Act is based on the EU Directives 93/42 EG and 98/79 EG, within the framework of which national law must comply. Quality assurance in medical laboratories in the established area is determined by an ordinance of the Medical Association (approved by the Ministry of Health). Quality assurance in medical laboratory analysis in the hospital sector is included in the Cure and Hospital Act. The ÖNORM K 1950 gives a qualified recommendation for the concrete implementation based on the international standard EN ISO 15189: 2014.

The relevant regulations on documentation for the traceability and liability of the medical laboratories are contained in part in the Medical Devices Act, in part in the Physicians Act and in the Health and Medical Institutions Act and are important for patients and authorities in order to be able to make inquiries and, if

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

necessary, assert claims. The structural and quality requirements laid down in the regulations are not only legally required for the correct medical high-quality performance of laboratories, but also one of the most important requirements for the work of clinically active doctors, who rely on medically meaningful findings and accompanying expertise for their work are instructed on the patient.

The Agency for Health and Food Security (AGES) is legally obliged (§68 MPG) to monitor all laboratories that use medical devices (in-vitro diagnostics) to ensure compliance with these legal provisions and to act accordingly in the event of violations to ensure that patients are at risk and can be prevented from the public by faulty laboratory analysis.

1.5.2. Assessment of diagnostic test

In the current pandemic situation, the test kits available on the market (in vitro diagnostics) have either been approved with urgent approvals for the American market (FDA) and then CE-marked by the manufacturers, or are only available as research kits for which there is no comprehensible verification the quality has been achieved by the manufacturer, whereby manufacturers can now only be monitored very incompletely, especially with SARS-CoV-2 kits, and are actually de facto autonomous when applying the CE label. This means increased caution is also required when carrying out CE-marked tests. CE-marked tests for SARS-CoV-2 diagnostics in the laboratory are, according to the current legal situation, not subject to the need for an objective evaluation of the manufacturer's information by an independent notified body.

For research kits, each laboratory must assume full responsibility for the analysis and clinical usability of the findings (in-house test), i.e. carry out the validation / evaluation itself, including acceptance and transport of the samples intended for the test, which are otherwise carried out by the industrial manufacturers in complex test procedures. The laboratory must document that the performance requirements in Annex I of EU Directive 98/79 EC have been met and that the documentation can be submitted to the authority if necessary.

The European Commission has summarized the current status of the literature on performance data from SARS-CoV-2 Test: "Current performance of COVID-19 test methods and devices and proposed performance criteria - Working document of Commission services" (Link: https://ec.europa.eu/docsroom/documents/40805">https://ec.europa.eu/docsroom/documents/40805).
This document also emphasizes that for many of the laboratory tests there are no independent studies to assess the performance data.

1.5.3. Validation and verification of laboratory tests

Every laboratory test that is to be used for diagnostic purposes on human samples must be checked in advance by the laboratory for its suitability. This also applies to CE-marked tests. The EN ISO 15189: 2014 standard differentiates between validation and verification of the test procedure.

Verification: validated testing methods from manufacturers (i.e. CE-marked tests) that are used without modification must be subjected to verification before being introduced into routine use. The performance characteristics of the test specified by the manufacturer must be verified by the laboratory, in particular it must be documented that the performance characteristics for the specific application in the respective laboratory are also achieved.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

Validation: Assessment of the performance characteristics of an examination procedure or laboratory test using a - usually more complex - evaluation procedure in the laboratory. Validation is used if there are no valid performance characteristics of an examination method validated by the manufacturer or if the method has been significantly modified in the laboratory. In particular, validation must be carried out for:

- non-standardized procedures (non-CE-marked test, especially all "research use only" tests or research kits);
- procedures designed or developed for the laboratory (in-house tests);
- standard procedures that are used outside their intended scope (CE-marked tests that are used for a purpose for which the manufacturer has not validated them);
- validated and then modified procedures (CE-marked tests that are used in the laboratory with significant modifications to the manufacturer's information).

1.5.4. Practical information

In the case of CE-marked laboratory tests for SARS-CoV-2, the manufacturer is obliged to provide the performance data of the test. The quality of this information varies widely among the tests currently available and is currently not being verified by an independent notified body. It is therefore the responsibility of the laboratory to check the quality of the performance data specified by the manufacturer and to assess whether the validation was carried out by the manufacturer according to scientific standards. In addition, the achievement of the performance data must be verified in the respective laboratory (see validation and verification of laboratory test). In practice, there are common problems with manufacturer information, which will be dealt with as examples.

1.5.5. Selection of subjects to ascertain clinical sensitivity and specificity

Ideally, clinical sensitivity and specificity of an examination procedure should be ascertained on a patient who has been coughed up, which is representative of the clinical question and also allows an assessment of the positive or negative predictive value of the test. In practice, due to the limitations of the test-independent definition of COVID-19 patients, almost all manufacturers use two independent patient cohorts to ascertain the clinical sensitivity and specificity. For example, the clinical sensitivity of an anti-SARS-CoV-2 antibody test in patients with PCR-related COVID-19 disease is assessed. Irrespective of this, sera from test persons who had been archived before the occurrence of SARS-CoV-2 are used to determine the specificity. This procedure makes sense in the current situation, but has certain limitations; in particular, no direct conclusions can be drawn from such studies that include the prevalence of SARS-CoV-2. It is expressly forbidden to use the sum of these two cohorts to calculate a positive or negative predictive value of the test, as is done by individual manufacturers. Such an approach would equate the prevalence of COVID-19 with that resulting from any combination of these two cohorts.

When assessing clinical sensitivity, it is particularly important that the patient cohort be representative of the respective question. If, for example, the clinical sensitivity of an anti-SARS-CoV-2 antibody test was tested exclusively in hospitalized patients in a late stage of the disease, it is inadmissible to apply these results to outpatients in the early phase of the disease at the onset of symptoms without further testing. Several manufacturers have therefore gone over to specifying the clinical sensitivity depending on the stage of the disease.

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

1.5.6. Insufficient number of cases and missing information about confidence intervals

„Current performance of COVID-19 test methods and devices and proposed performance criteria - Working document of Commission services" (Link: https://ec.europa.eu/docsroom/documents/40805), emphasizes the need for 95% confidence intervals for the Specify the results of diagnostic sensitivity and specificity of a laboratory test, which was examined in a clinical study using a suitable cohort. In this context, the number of cases of the examined subjects is significant. Figure 1 shows the relationship between the number of cases and the width of the confidence interval in a test with 95% specificity according to Bruderer (**https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1553-2712.1996.tb03538.x**).

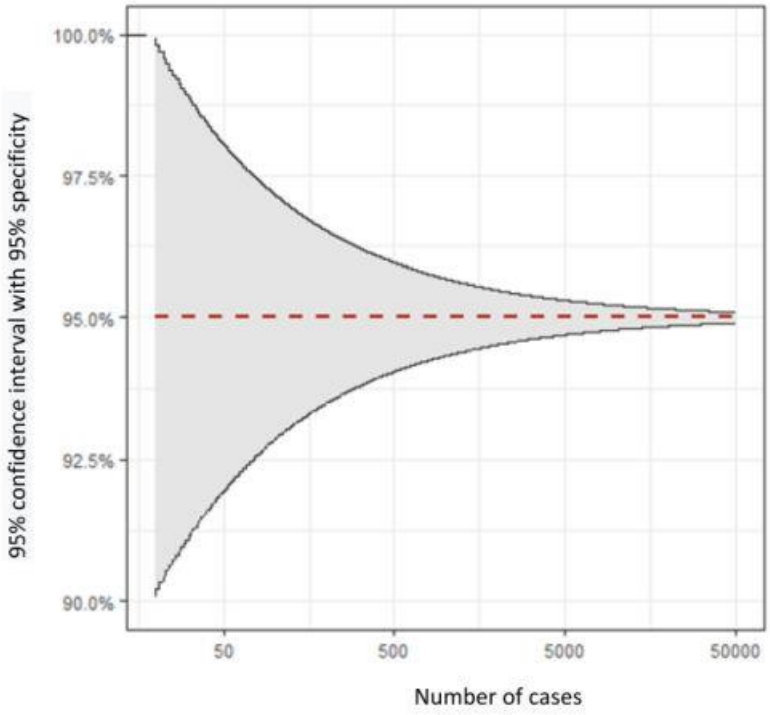

Figure 1: Example of the relationship between the width of the 95% confidence interval and the number of cases, with the following assumptions: 95% specificity, error probability α=0.05, 1% prevalence

For example, if a manufacturer checks the diagnostic specificity of an Anti-SARS-CoV-2 antibody test using only 50 subjects and no false positive result is shown for these 50 samples, the validity of this test evaluation is relatively low, although the specificity is nominally 100% . Individual manufacturers have evaluated anti-SARS-CoV-2 antibody tests in more than 1,000 subjects, which leads to more statistically meaningful results with a narrow confidence interval.

1.5.7. External quality controls

Round robin tests are an essential means of external quality assurance. For this purpose, samples are sent from an external point to the participating laboratories. The interlaboratory test samples are to be used and processed in the laboratory like patient samples. The results are reported back by the laboratory and assessed by the proficiency testing manager. Both SARS-CoV-2 PCR tests and anti-SARS-CoV-2 antibody tests are now available in round robin tests from different providers. The interlaboratory test samples are to be used and processed like patient samples. The results are assessed by the proficiency testing management. The ÖGLMKC advocates mandatory participation in

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

proficiency testing for SARS-CoV-2 tests. For all laboratories that carry out SARS-CoV-2 analyzes on humans without exception, sanctionable regulations to ensure the required quality in the use of in-vitro diagnostics are essential.

1.5.7.1. Assessment of results in SARS-CoV-2 PCR round robin tests

Published data from the INSTAND round-robin test SARS-CoV-2 virus genome detection April 2020 have led to speculations about the specificity of the SARS-CoV-2 PCR. In this round-robin test, 1.4% false positive results were reported for negative samples. However, the data from a round-robin test cannot be applied directly to the specificity data of an assay; rather, probes reflect the quality of the participating laboratories. This 1.4% relates to a few laboratories that have reported incorrect results. According to the assessment of the ÖGLMKC and the ÖQUASTA, this does not correspond to a random distribution, but is mainly due to problems with sample handling, test execution or the input of results in the respective laboratory. In this sense, round-robin tests are particularly important in order to identify problems in the respective laboratory and then to re-evaluate the entire process in the laboratory from the sampling to the output of findings for possible sources of error. In no way can a general statement on the specificity of SARS-CoV-2 PCR tests be derived from this. In real operation of large, high-quality medical laboratories, false positive PCR results are an absolute rarity and the specificity of SARS-CoV-2 PCR tests is> 99.9% according to internal evaluations of screening examinations taking into account additional test results of the people [Von This extremely low technical false-positive rate has to be differentiated asymtomatic persons with a low viral load, who can be recognized in screening examinations (see 1.1.4.3.)].

False negative results are frequently observed in samples with low virus levels in round trials (Görzer et al. Journal of Virology. Doi: 10.1016 / j.jcv.2020.104537). This may indicate that the test used is not sensitive enough to reliably identify samples with a low viral load as positive. For such laboratories and tests, the ÖGLMKC assesses the use of pool testing as critical.

Such interlaboratory test results clearly show how important the quality of the testing laboratories is. Against this background, the ÖGLMKC is critical of the current legal situation that allows non-medical laboratories to carry out SARS-CoV-2 tests in the context of the COVID-19 pandemic on the basis of the Epidemic Act. Trained specialists, extensive experience with molecular infection diagnostics, effective systems of quality assurance and medical reports are basic requirements to ensure high quality care in Austria. The highest medical quality standards must be uncompromisingly demanded by laboratories that carry out SARS-CoV-2 PCR tests.

2. General laboratory diagnostics for COVID-19

2.1. Handling blood samples and other bodily fluids of COVID-19 patients

In addition to respiratory secretions, coronavirus SARS-CoV-2 was also detected in the stool and in isolated cases in urine, blood/plasma and cerebrospinal fluid by means of PCR. Although no contagion via these bodily fluids (urine, blood/plasma and cerebrospinal fluid) is documented, such a must not be ruled out with the last certainty at present. Based on the current data situation, ÖGLMKC therefore recommends that general hygiene and protective measures be taken into account when working with blood samples from COVID-19 patients and that unnecessary aerosol formation during sample handling

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

should be avoided. For stool samples of COVID-19 patients, the potential risk of infection is currently assessed higher, so the ÖGLMKC recommends reducing the analysis of stool samples from COVID-19 patients or suspected cases to an absolutely necessary minimum and taking additional protective measures to minimize the risk of potential infection. In the course of a laboratory request, the laboratory must in principle be notified by the sender that the submission is samples of a COVID-19 patient or a COVID-19 suspected case.

In detail, we allow ourselves to refer to the recommendations for handling test material from Covid 19 positive / suspect patients in the laboratory of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP):
Link:
https://www.oeghmp.at/media/empfehlungen_zum_umgang_mit_untersuchungsmaterial_von_covid-19-positiven-verdaechtigen_patienten_im_labor.pdf

2.2. Value of laboratory parameters in COVID-19

Clinical and laboratory data showed that some laboratory values are frequently changed in COVID-19 (see summary in Lippi G, Plebani M. Clin Chim Acta. 2020 Mar 4. https://doi.org/10.1016/j.cca.2020.03.004). Among the hematological parameters, it was found that lymphopenia in particular is associated with the severity of the course of the disease. Patients suffering from COVID-19 with lymphocytopenia had a poor prognostic course (Tan L et al. Signal Transduct Target Ther. 2020 Mar 27; 5:33. https://doi:10.1038/s41392-020-0148-4). In most cases, there was a reduction in CD8 positive cytotoxic T suppressor cells (Liu Y et al. Sci China Life Sci. 2020 Mar; 63: 364–374. https://doi.org/10.1007/s11427-020-1643-8; Velavan TP et al. Int J Infect Dis 2020 Jun; 95: 304-307. https://doi:10.1016/j.ijid.2020.04.061).

Patients with severe disease had more frequent liver dysfunction with increased alanine (ALT) and aspartate aminotransferases (AST) levels compared to mild forms (Zhang C et al. Lancet Gastroenterol Hepatol. 2020 May; 5: 428-430. https://doi:10.1016/S2468-1253(20)30057-1). The severity and prognosis of the course of the disease showed a clear association with increased inflammatory markers, in particular the C-reactive protein (CRP), procalcitonin and ferritin (Terpos E. Am J Hematol. 2020 Jul; 95: 834-847. https://doi:10.1002/ajh.25829).

In a current study, a D-dimer = 2.0 µg / mL (4-fold increase) at the time of hospitalization proved to be a predictive mortality marker (Zhang L. J Thromb Haemost. 2020 Jun; 18: 1324-1329. https://doi:10.1111/jth.14859). Compared to healthy people, COVID-19 sufferers showed significantly increased D-dimer and fibrinogen levels as well as greatly reduced antithrombin levels as a sign of impaired blood clotting (Han H. Clin Chem Lab Med. 2020 Jun 25; 58: 1116-1120. https://doi:10.1515/cclm-2020-0188). An elevated troponin value turned out to be a predictive marker for mortality in patients with COVID-19 pneumonia (Du RH. Eur Respir J. 2020 May 7; 55: 2000524. https://doi:10.1183/13993003.00524-2020).

Some studies have also examined the importance of cytokine determination in the treatment of COVID-19 patients. In particular, IL-6 and TNF-alpha were identified as independent and prognostically relevant biomarkers for patients. The study authors recommend that these cytokine determinations should be

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

taken into account for the management of patients and the guidance of therapies (DM Del Valle, PMID: 32511562).

With regard to the value of laboratory diagnostic parameters in clinical decision-making, further large data sets and meta-analyzes are necessary. The ÖGLMKC recommends a structured collection and evaluation of laboratory data from Austrian COVID-19 cases, taking into account previous publications. This is intended to bundle the experiences of the various health facilities and to subject the prognostic significance of the individual biomarkers to a further evaluation.

3. Further literature

Federal Ministry of Social Affairs, Health, Care and Consumer Protection
https://www.sozialministerium.at/Informationen-zum-Coronavirus/Neuartiges-Coronavirus-(2019-nCov).html

Agency for Health and Food Safety (AGES)
https://www.ages.at/themen/krankheitserreger/coronavirus/

Robert Koch Institute (RKI)
https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/nCoV.html?cms_box=1&cms_current=COVID-19+%28Coronavirus+SARS-CoV-2%29&cms_lv2=13490882

World Health Organization (WHO)
https://www.who.int/emergencies/diseases/novel-coronavirus-2019

Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP)
https://www.oeghmp.at/

4. Contact details of laboratories for the detection of SARS-CoV-2 in Austria

The ÖGLMKC has launched a website of specialist medical laboratories for SARS-CoV-2 diagnostics, which lists detailed information on the methodology used and contact data of the laboratories and offers a search and filter function. https://www.covid19-labore.at/

You can also find other laboratories. See the homepage of the Agency for Health and Food Safety (AGES) https://www.ages.at/themen/krankheitserreger/coronavirus/

5. Other

Note for laboratories :
Interested specialist laboratories who agree to the publication of their contact details on the website mentioned above, please contact the ÖGLMKC office. You can find more information on the website.
https://www.covid19-labore.at/

All laboratories that carry out SARS-CoV-2 diagnostics are urged to take part in round trials as part of external quality assurance. In Austria, the Austrian Society for Quality Assurance and Standardization of Medical Diagnostic Examinations (ÖQUASTA) offers round-robin tests for SARS-CoV-2 diagnostics.
https://oequasta.at/

Geschäftsstelle der ÖGLMKC: Infomedica KG, Tullnertalgasse 72, 1230 Wien
Tel 0699/1011 7657; office@oeglmkc.at; öglmkc.at

Authors in alphabetical order :

- **Christoph Binder**, Clinical Institute for Laboratory Medicine, Medical University of Vienna
- **Dietmar Enko**, Institut für Medizinische und Chemische Labordiagnostik, Landeskrankenhaus Hochsteiermark & Klinisches Institut für Medizinische und Chemische Labordiagnostik, Medizinische Universität Graz
- **Markus Exner**, Labors.at - Mühl-Speiser-Bauer-Spitzauer and partner specialist for med. and chem. Laboratory diagnostics OG, Vienna
- **Georg Greiner**, Clinical Institute of Laboratory Medicine, Medical University of Vienna
- **Andrea Griesmacher**, Central Institute for Medical and Chemical Laboratory Diagnostics, University of Innsbruck
- **Alexander Haushofer**, Institute of Medical and Chemical Laboratory Diagnostics, Klinikum Wels-Grieskirchen
- **Gregor Hörmann**, Central Institute for Medical and Chemical Laboratory Diagnostics, University of Innsbruck & Clinical Institute of Laboratory Medicine, Medical University of Vienna
- **Harald Kessler**, Diagnostics & Research Institute for Hygiene, Microbiology and Environmental Medicine, Medical University of Graz
- **Georg Mustafa**, Medilab Medical - Chemical Laboratory Dr. Mustafa Dr. Richter OG, Salzburg
- **Manfred Nairz**, Department of Internal Medicine II & Central Institute for Medical and Chemical Laboratory Diagnostics, Innsbruck University
- **Paul Niedetzky**, myLab Labor Dr. Niedetzky, Linz
- **Mathias M. Müller**, President of ÖQUASTA
- **Thomas Perkmann**, Clinical Institute for Laboratory Medicine, Medical University of Vienna
- **Matthias Perné-Mayerhofer**, Medical Laboratory DDr. Johann Perné, Klagenfurt
- **Franz Ratzinger**, Ihr Labor ordination community for laboratory diagnostics and microbiology, Vienna
- **Christian Schweiger**, Clinical Institute for Laboratory Medicine, Medical University of Vienna
- **Robert Straßl**, Clinical Institute of Laboratory Medicine, Medical University of Vienna
- **Thomas Szekeres**, Clinical Institute of Laboratory Medicine, Medical University of Vienna
- **Andreas Tiran**, Laboratory Dr. Tiran, Graz

Correspondence:

Gregor Hörmann
Central Institute for Medical and Chemical Laboratory Diagnostics, University Hospital Innsbruck & Clinical Institute for Laboratory Medicine, Medical University of Vienna
Postal address: Universitätskliniken Innsbruck, ZIMCL, Anichstraße 35, 6020 Innsbruck
Tel: +43 (0) 512 504 - 83673
E-Mail: gregor.hoermann@tirol-kliniken.at; gregor.hoermann@meduniwien.ac.at