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i3 innovation inspiration information
Immundiagnostik AG

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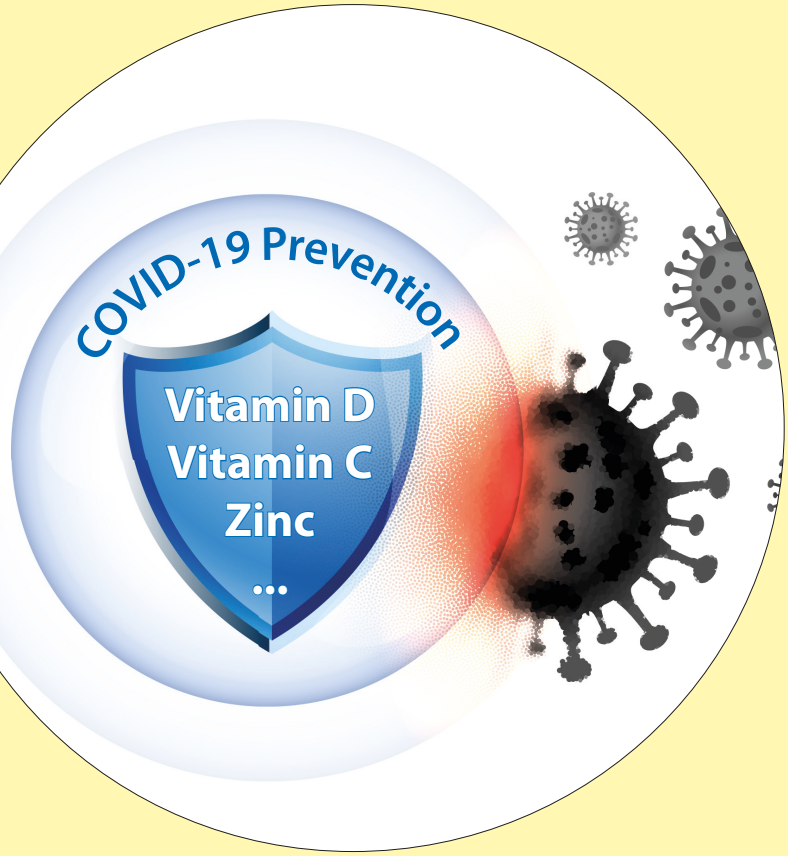
Special editon:

COVID-19 Prevention



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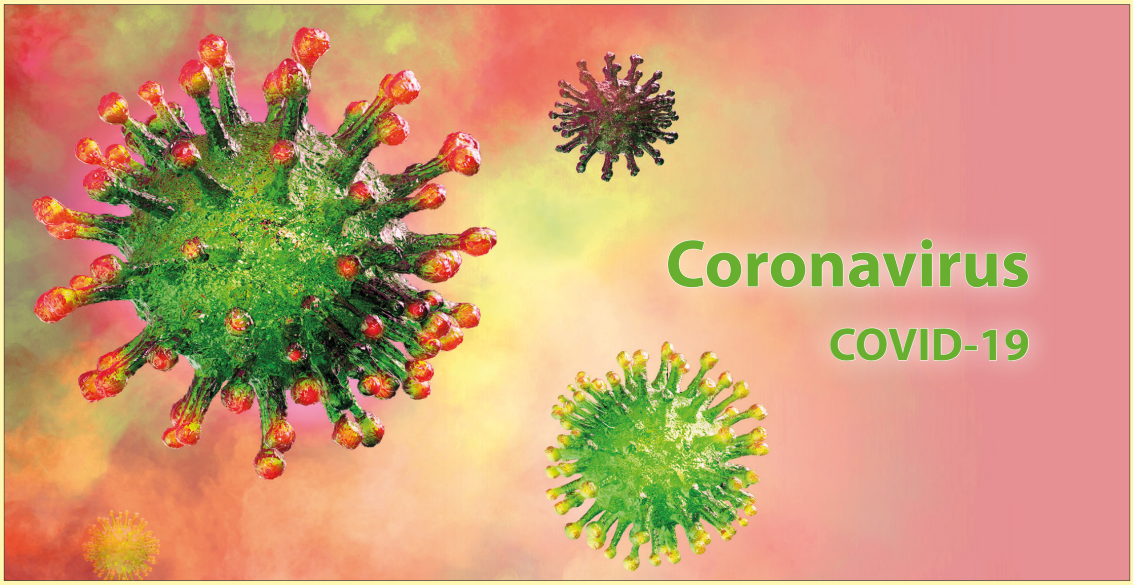
Detection of SARS-CoV-2



➲ **PCR: MutaPLEX® Coronavirus**

Detection of an acute infection

➲ **ELISA IgM and ELISA IgG: SARS-CoV-2**

Detection of an induced immune response

CE marked

immun diagnostik

EDITORIAL

Dear Readers,

based on the current situation, we have set the focus of this special issue of **i³** innovation inspiration information on **COVID-19 prevention**.

We perceive the fewer visits of sick patients to clinics, even hospitals are announcing reduced hours. Your laboratories are receiving significantly less samples. However, right now health status testing is crucial – **as those who have sufficient vitamins and micronutrients, have a reduced risk of infection.** Especially persons belonging to known risk groups who often exhibit significantly decreased concentrations of micronutrients in blood.

However, a deficiency is easy to confirm in the laboratory. After being infected with SARS-CoV-2, people with a deficient supply of micronutrients have an increased risk for a severe course of the COVID-19 disease. Modified supplementation for individuals with missing vitamins and micronutrients can possibly prevent a severe course, alleviate secondary disease, and save lives!

The most important candidates include **vitamin C, vitamin D, and zinc:** All three have protective qualities which can reduce the susceptibility to infection in general and also to bacteria and viruses as SARS-CoV-2. In some countries, vitamin C is already being given as an adjuvant therapy for COVID-19.

As a qualified laboratory, now is the time to offer testing of health status: In this way – should a second wave of infection occur – you would disburden the health system through preventive measures, increase the chances of positive outcome of an infection, and save lives.

We would like to present a selection of current interesting publications and, thereby, assist you again in receiving more laboratory requests.

Please stay healthy.

Dr. Sonja Bastian
Editorial office i³ newsletter

Maintaining health status!

Fewer check-ups or office visits for minor complaints because of fear of a COVID-19 infection

↓

Current treatments are mainly for suspicion of COVID-19 infection (in medical practices)

↓

50% fewer laboratory requests for health status of the population (nutritional status, risk factors, etc.)

↓

The population's health status is the most important component in the infection and severe course of COVID-19 disease prevention.

SARS-CoV-2 and COVID-19

The role of vitamins in the treatment of COVID-19

Researchers are looking at different methods and concepts for the treatment of the new coronavius SARS-CoV-2 infections and the alleviation of the course of the disease caused by the virus. For this, sufficient knowledge and current experiences with the disease is fundamental. The following article gives a detailed overview of the status of the current knowledge and presents vitamin C and D as interesting protective and effective substances.

Publication:
Kakodkar P, Kaka N, Baig MN (2020)
A Comprehensive Literature Review on the Clinical Presentation, and Management of the Pandemic Coronavirus Disease 2019 (COVID19).
Cureus 12(4) e7560. DOI: 10.7759/cureus.7560

SARS-CoV-2: Biochemistry, epidemiology and clinical appearance

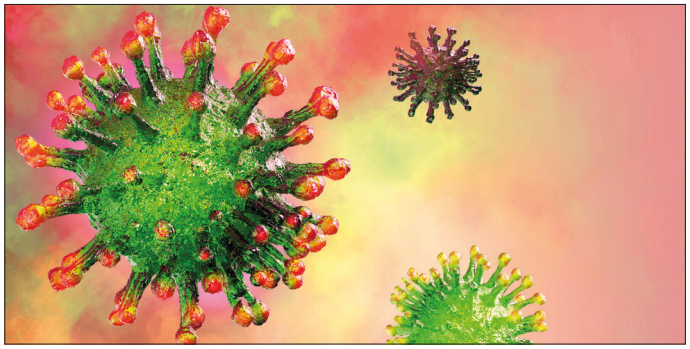
On January 7, the CDC (Center of Disease Control) in China isolated a new coronavirus and labelled it „2019-nCoV", also called „Wuhan Coronavirus". The WHO changed the name to SARS-CoV-2 to separate the pandemic virus from the geographic and country descriptions and to emphasize the SARS disease (severe acute respiratory syndrome). SARS-CoV-2 is closely related to the SARS-CoV-1 from 2002. In addition, there are numerous other coronaviruses that cause harmless common colds.

Corona viruses are enveloped RNA viruses that are surrounded by characteristic, spikey glycoproteins that form a type of crown (Latin: *corona*) which is responsible for the nomenclature. Coronaviruses currently have the largest know single stranded RNA genome.

An important structural protein of coronaviruses is the spike glycoprotein S, consisting of three S1-S2 heterodimers and which binds to the *angiotensin-converting enzyme 2* (ACE) receptor of type II pneumocytes. The virus enters lung cells via endocytosis. In the process, it separates from the envelope and begins replication in the cytoplasm of the host cell. The newly formed nucleocapsids finish their construction with the addition of a new envelope by budding through the membrane into the endoplasmic reticulum of the cell. They are distributed further from there. The massive protein biosynthesis finally leads to apoptosis of the infected cells.

The infection leads to a "cytokine storm" (hypercytokinemia), where excessive amounts of cytokines are produced, leading to attraction and activation of neutrophils. This leads to the destruction of capillary and alveolar walls allowing fluids to enter alveoli.

Similar to influenza viruses, SARS-CoV-2 is spread by aerosols, droplets, and contact with infected persons. Therefore, it is important to practice hand hygiene, avoiding droplet infection



(mouth and nose protection), as well as "social distancing", effective measures to reduce the spread. The long incubation period compared to other viruses is a problem. This leads to a quick spread of the infection and a world-wide pandemic.

Virus family	Virus type (disease)	Incubation period
Corona viruses	SARS-CoV-2 (COVID-19)	2–14 days
	SARS-CoV-1 (SARS)	2–7 days
	MERS-CoV (MERS)	5 days
Orthomyxovirus	H1N1 (swine flu)	1–4 days
	Influenza (flu)	2 days

Table 1: Incubation periods of different viurs diseases

The clinical manifestation of the SARS-CoV-2 infection is called *Coronavirus Disease* (COVID-19) where "19" designates the year 2019, when the first cases were seen in Wuhan. Persons infected with COVID-19 show the following symptoms with the respective frequencies:

Symptom	Frequency
High fever	85,0%
Cough	67,7%
Fever	45,0%
Sputum	33,4%
Dyspnea	18,6%
Muscle and joint pain	14,8%
Sore throat	13,9%
Headache	13,6%
Chill	11,4%
Nasal congestion	4,6%

Table 2: Symptoms of COVID-19 and their frequency of occurrence

SARS-CoV-2 and COVID-19

A severe infection of the lung can lead to ARDS (*acute respiratory distress syndrome*) and even to septic shock. Both situations require intensive care treatment.

Persons at risk show an increased amount of ACE2 receptors (ACE2R). This facilitates the entry of the viruses into cells and can thereby lead to a severe course of disease. The risk groups include persons with coronary heart disease, high blood pressure, diabetes, and also those with preexisting conditions such as cancer, COPD, hepatitis and liver cirrhosis, kidney insufficiency and gastrointestinal diseases.

Current assumptions are that Asians have a higher expression of ACE2R than Caucasians and Afro-Americans, explaining the heavy spread in Asian regions.

The role of vitamins

Vitamin C (L-ascorbic acid), given intravenously at higher doses, has a protective effect in the treatment of sepsis resulting from ARDS. It supports the maintenance of the barrier function of the alveolar epithelium and regulates the fluid level of the alveoli with the increased expression of protein channels (e.g., aquaporin, Na^+/K^+ ATPase). High-dose intravenous treatment with vitamin C decreases cell-free DNA and thereby reduces the concomitant systemic infections and sepsis induced multi-organ failure. In February 2020, Zhongnan Hospital in southern China started a clinical study (NCT04264533), to confirm the effectiveness and safety of vitamin C in COVID-19 pneumonia.

Vitamin D strengthens the immune system and supports the regeneration of endothelial cells. These characteristics could be used to reduce lung injury.

In addition, vitamin D protects against bacterial and viral respiratory illnesses: protection increases by 70% if a deficiency (<25 nmol/l) is offset with supplementation.

Did you know?

The **gold standard** for the diagnosis of COVID-19 is **real-time reverse transcription polymerase chain reaction** (real time RT-PCR) with a sensitivity of 66–80%, which means that 20–34% of those infected, tested negative. The reason is that many people are in early stages of illness development and the viral load is therefore still too low to be detected. A single, negative RT-PCR does not automatically mean that no infection is present. Therefore, RT-PCR should be repeated 24–72 hours following a negative result.

Most COVID-19 patients have normal or decreased leucocyte count or lymphocytopenia. In addition, an increase in pyrogenic cytokines, such as IL-6, IL-10, and TNF-α can be demonstrated. In particular, patients requiring intensive care often have increased plasma levels of interleukins (IL-2, IL-7, and IL-10) and other cytokines.

Neutrophilia and increased levels of D-dimer concentrations as well as increased concentrations of creatinine were seen in the critically ill.

Patients are considered recovered when they no longer have a fever and the respiratory symptoms are gone, no abnormalities are visible on a CT, and two RT-PCR tests within a 24-hour period are negative.

Products for the diagnosis of SARS-CoV-2:

➲ MutaPLEX® **Coronavirus**	KG192696	qualitative Real-Time-RT-PCR (96 Tests)
	KG1926-384	qualitative Real-Time-RT-PCR (384 Tests)
	KG1926-768	qualitative Real-Time-RT-PCR (768 Tests)

Vitamin D and COVID-19

Vitamin D supplementation to minimize the risk of COVID-19

Vitamin D, produced in the skin by UVB rays or ingested orally via nutrients, is metabolized in the liver to 25(OH)D and mainly in the kidney, and in other organs as needed, to the hormonally active 1,25(OH)$_2$D (calcitriol) (refer to Figure 1).

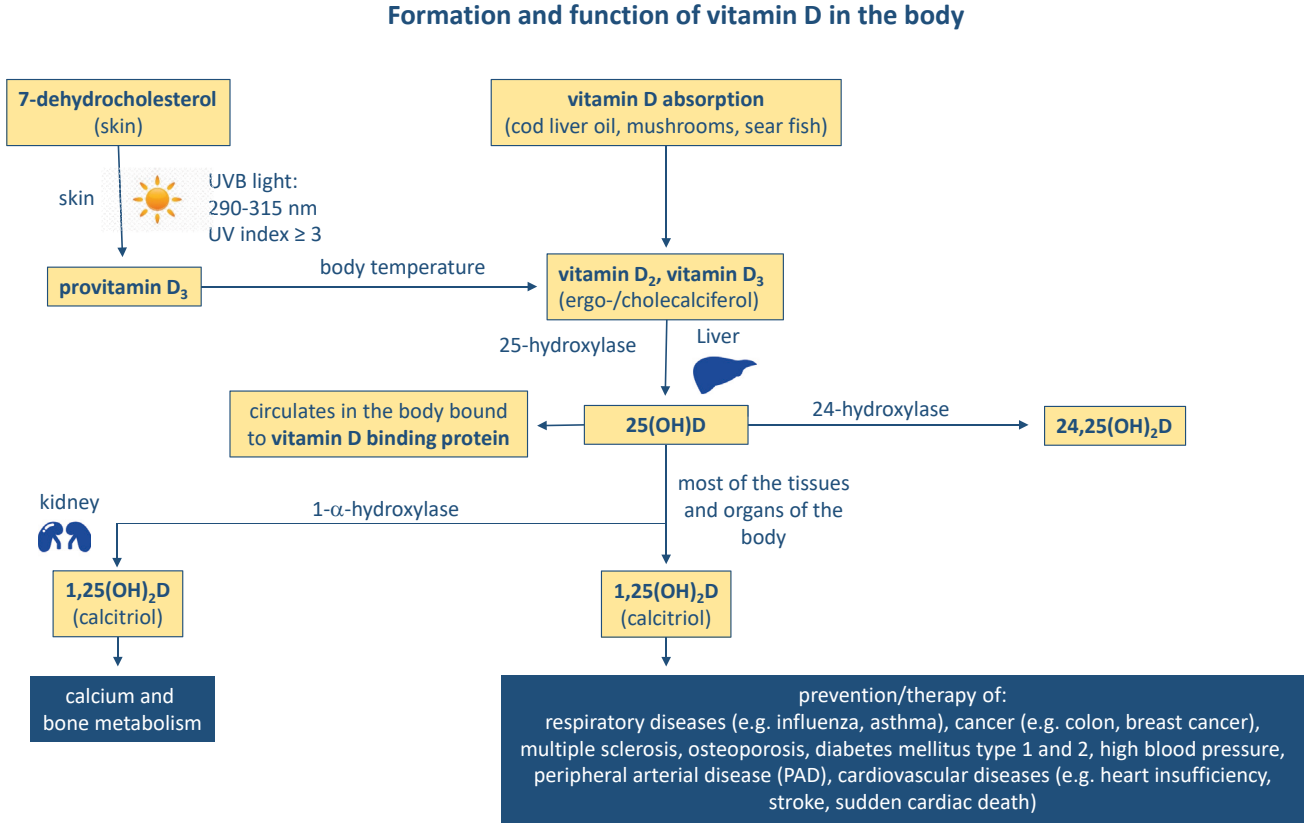


Figure 1: according to Gröber U (2020) Vitamin D: Vom Sonnenvitamin zum Sonnenhormon, Zs. f. Orthomol. Med 18: 28-32

The main effect of calcitriol is the binding to nuclear vitamin D receptor, a transcription factor. However, the most well-known effect of calcitriol is the regulation of the calcium concentration in serum in the feedback loop with parathormone (PTH).

Different effects could also be ascribed to vitamin D in the control of infections, summarized in the following table:

Parameter	Effect on	Reaction
1,25-(OH)$_2$ D and defensins	Innate immune system	Formation of antimicrobial peptides (cathelicidin, LL-37) to kill pathogens that have infiltrated (cathelicidin: gram-positive/ gram-negative) and to prevent the replication of viruses (LL-37: Dengue Virus)
Vitamin D	Cellular immunity	Decrease in the production of pro-inflammatory Th1-cytokines (TNF-α, IFN-γ) Increase in the expression of anti-inflammatory cytokines
1,25-(OH)$_2$ D	Acquired immune system	Formation of pro-inflammatory cytokines (IL-2 and IFN-γ) is suppresssed, which then decreases the response of T-helper cells type 1 (Th1). Th2 cells produce an increased amount of cytokines, which further reduce the activity of Th1 cells. Regulatory T-cells are activated and suppressed in inflammatory processes.

Table 3: Effect of vitamin D and its metabolites on different parts of the immune system.

Based on experiences in the area of influenza treatment, there is evidence that supplementation with vitamin D could also have a positive effect on the course of COVID-19 disease:

1. The outbreak occurred in winter. The 25(OH) vitamin D concentration (25(OH)D) is lowest at this time of year.
2. Vitamin D deficiency promotes the emergence of the development of *Acute Respiratory Distress Syndrom* (ARDS).
3. Schwere Verläufe findet man häufiger bei Älteren und Patienten mit Vorerkrankungen, die meist einen Vitamin-D-Mangel aufweisen.

In the following, we present an article regarding the reduction of risk in influenza and COVID-19 using a suggested prophylactic treatment of 10,000 IU/d of vitamin D_3 for several weeks, to quickly increase the concentration of 25(OH)D to a target level of 40–60 ng/ml (100–150 nmol/l). In patients (ill) with COVID-19, even higher doses of vitamin D_3 seem to be appropriate. Further studies on the prevention and treatment options are advisable with regard to a possible second wave.

Publication:

Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, Bhattoa HP (2020)
Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths.
Nutrients 12(4) pii 988; doi:10.3390/nu12040988

Vitamin D in the control of infections

COVID-19 is associated with the production of pro-inflammatory cytokines, C-reactive protein (CRP), an increased risk of pneumonia, sepsis, ARDS, and heart insufficiency.
The Case Fatality Rate (CFR) in China was 6–10% for persons with cardiovascular disease, chronic respiratory disease, diabetes, and high blood pressure. In addition to preexisting diseases, the CFR was also high in areas with severe air pollution (China, northern Italy).
Results of several clinical studies show a connection between these preexisting diseases and insufficient vitamin D (25(OH)D): Severe illnesses with pneumonia, increased proinflammatory cytokines (IL-6), increased serum CRP, increased risk of developing sepsis, ARDS, heart insufficiency, and diabetes mellitus are associated with low serum levels of 25(OH)D.

Clinical and epidemiology features of COVID-19

Due to the low levels of UVB rays in winter, the self-synthesis of vitamin D is reduced. This favors all types of infections, e.g. *respiratory syncytial virus* (RSV), influenza viruses, and SARS-CoV. In the tropics, this seasonality is associated with the rainy season.
Different studies report that in the chronically ill the 25(OH)D concentrations are less than for healthy people. Persons with COPD, pneumonia or high blood pressure can have 25(OH)D concentrations of 13–16 ng/ml, which represents a minor vitamin D deficiency (10–20 ng/ ml).

Clinic	Association with 25(OH)D
Beginning of the epidemic in the winter of 2019 (low levels of UVB rays)	Low levels of 25(OH)D in winter
Incidence and CFR is higher in men than in women	Smoking reduces 25(OH)D in serum (men smoke more frequently in China than women)
Risk factor „age“	Chronic illnesses are more frequent in older patients; a sufficiently high level of vitamin D reduces the risk for chronic illnesses
Risk factor „diabetes“	Diabetics have a low level of 25(OH)D, which favors the susceptibility to infection
Risk factor „high blood pressure and cardiovascular disease“	A low level of 25(OH)D favors the susceptibility to infection
Risk factor „chronic respiratory disease“	The level of 25(OH)D is inversely correlated with the risk, the severity, and the course in COPD patients
Risk factor „air polution“	Air pollution is directly associated with lower levels of 25(OH)D

Table 4: Vitamin D status (25(OH)D) in comparison with clinical and epidemiological data

Coronaviruses, that cause acute respiratory syndrome, damage the lung due to the increased production of cytokines produced by Th1 cells. This is the beginning of the „cytokine storm“, a reaction to infection by the virus, leading to serious courses for SARS-CoV and MERS-CoV. In contrast, in SARS-CoV-2 infection, in addition to Th1 cytokines, anti-inflammatory Th2 cytokines are also produced.

Vitamin D and COVID-19

Treatment recommendation

The main season for virus infections is the time of year in which the levels of 25(OH)D are the lowest (winter, rainy season). Supplementation should ideally begin several months prior to reach a sufficient level of 25(OH)D and to be better protected against infection.

Disease	Target level of 25(OH)D
Acute respiratory infections	20–30 ng/ml
Pneumonia (Community acquired)	38 ng/ml
Optimal protection	40–60 ng/ml (100–150 nmol/l)

Table 5: Target level of vitamin D for protection against diseases

A level of 40–60 ng/ml of 25(OH) vitamin D provides optimal protection, which can be supplemented with 2000–5000 IU/d Vitamin D_3 if a deficiency is present. In addition to the vitamin D dose, a sufficient amount of magnesium should be available, since all enzymes that metabolize vitamin D need magnesium as a co-factor. A daily dose of 250–500 mg magnesium in combination with 500–1000 mg calcium is recommended.

Further studies are needed on the effectiveness and safety of vitamin D in the prevention of COVID-19 and to avoid severe and fatal courses.

Products for the determination of vitamin D:

➲ **1,25-$(OH)_2$-Vitamin D**	K 2112	ELISA
➲ **1,25-$(OH)_2$-Vitamin D_3/D_2**	KM1000	*IDK*® ImmuTube LC-MS/MS Kit
	KM1101	*IDK*magnetics®
➲ **25-OH-Vitamin D**	K 2010	CLIA
	K 2020	ELISA
	K 2020DBS	ELISA (dried blood)
➲ **25-OH-Vitamin D_3 / D_2**	KC3000	HPLC Reversed Phase
	KM5000	LC-MS/MS
➲ **24,25-$(OH)_2$-Vitamin D_3 / 25-OH-Vitamin D_3/D_2**	KM1300	LC-MS/MS
➲ **Vitamin D binding protein**	K 2314	ELISA

Further products for the determination of micronutrients:

➲ **Calcium** *(RUO)*	KJCCA030	colorimetric
➲ **Magnesium** *(RUO)*	KJCMG035	colorimetric

Vitamin C for the adjuvant therapy of COVID-19

The term „Vitamin C" refers to L-ascorbic acid and its derivates, which all possess similar biological functions. Plants and animals can form vitamin C from glucuronic acid. Humans, great apes, bats, guinea pigs, and several other animal species are not able to do this as the last enzyme in the synthesis path – L-gulonolactone oxidase – is missing. Instead, these species have a glucose transport (GLUT) on their erythrocytes, that selectively take up vitamin C into the erythrocytes and store it. This reduces the daily need in comparison to the self-synthesizing species, which also do not have storage capacities. Vitamin C exhibits antioxidative effects which are based on its characteristic as a reduction agent. In addition, it is an important factor in carnitine synthesis and, via the ***hypoxia inducible factor*** *(HIF-1α), has an influence on transcription of proteins needed for the maintenance of iron homeostasis, angiogenesis, and cell proliferation.*

Effect of vitamin C	Enzymes / Mechanisms	Use
Activation	Hydroxylases	Formation of collagen
	4-Hydroxyphenylpyruvat-Dioxygenase	Tyrosine degradation → fumarate and acetoacetate
Co-Factor	Cholesterol-7-Hydroxylase	Cholesterol and bile acid synthesis
	Lysine/methionine metabolism	Synthesis of carnitine
	Ascorbic acid dependent amidation	Formation of neuro-endocrine hormones (gastrin, bombesin, CRH, TRH)
	Peptidylglycine Monooxygenase-alpha amidase	Synthesis of vasopressin
Promotion	Uptake of trace elements	Iron resorption in the colon
Stimulation	Cytochrome P450	Detoxification of the liver
Inhibition	Protein glycosylation	Diabetes prophylaxis
	Formation of nitrosamine in the stomach	Effect as antioxidant
Hydrogen donor	Dopamine monooxygenase	Formation of noradrenaline and adrenaline

Table 6: Effect and use of vitamin C in the human body

Publication:

Hernandez A, Papadakos PJ, Torres A, Gonzales DA, Vives M, Ferrando C, Baeza J (2020)
Two known therapies could be useful as adjuvant therapy in critical patients infected by COVID-19.
Rev Esp Anestesiol Reanim Apr 14. pii: S0034-9356(20)30075-X.
doi: 10.1016/j.redar.2020.03.004

COVID-19 can develop into a severe disease with dyspnea and reduced oxygen saturation, and in more than a third of the cases – about 8 days following onset of symptoms – a support of ventilation is required. Mortality varies depending on the patient group and runs between 3% and 20%. It is therefore possible that COVID-19 is made up of a heterogenous population of viral subgroups with different degrees of virulence which would explain the regional and temporary varying courses.

Pharmacokinetics

Vitamin C fulfills most of its biological function intracellularly and is taken up with the participation of specific membrane transporters. The absorption, distribution, and retention of vitamin C is mainly determined by the family of sodium dependent vitamin C transporter (SVCT). Expression patterns and local concentrations of the transporter in the body lead to highly complex, compartment dependent, non-linear pharmacokinetics of vitamin C at physiological concentrations. When given intravenously, however, the pharmacokinetics of vitamin C changes and shows a dose-dependent constant half-life.

Vitamin C circulates in plasma, is filtered through renal glomeruli, and is resorbed in the proximal tubules via the sodium dependent vitamin C transporter 1 (SVCT1). SVCT1 regulates vitamin C homeostasis for the whole body. The sodium dependent vitamin C transporter 2 (SVTC2) is a transporter with high affinity, but limited transport capacity. It protects metabolically active cells from oxidative stress, by enabling the enrichment of vitamin C where it is needed. Dehydroascorbic acid (the oxidized form of vitamin C) is transported by the family of glucose transporters (GLUT). Meanwhile, it is reduced to prevent an irreversible degradation.

During massive inflammation processes, for example sepsis, the uptake of vitamin C into cells is reduced due to the increased release of cytokines.

Vitamin C and COVID-19

Biological effect of vitamin C

The known physiological and biochemical actions of vitamin C can be traced to its action as an electron donor.

Vitamin C has immune stimulating, antioxidative, anti-inflammatory, antiviral, and possibly antimutagenic effects.

Vitamin C is proven to improve chemotaxis of neutrophils, phagocytosis, and therefore microbial clearance.

Clinical applications

A study on heart surgery patients showed that preoperative administration of vitamin C was able to reduce renal insufficiency induced by the anesthetic etomidate. The vitamin C dependent synthesis of the vasopressors noradrenaline and vasopressin plays an important role in supporting cardiovascular function during a severe infection and septic shock.

A current study uses a combination of vitamin C (1.5 g i.v. every 6 hours), hydrocortisone (50 mg i.v. every 6 hours), and thiamine (200 mg i.v. every 12 hours) **for the treatment of sepsis.** Patients treated with this procedure showed a **greater than 30% reduced overall mortality** even with preexisting comorbidities and mortality risk predicted prior to treatment. Currently, there are several ongoing, randomized and controlled clinical studies, including the VICTAS, ACTS, and HYVCTTSSS studies, with the goal of confirming the positive effects of vitamin C and other supplements in critically ill patients with sepsis.

Acute respiratory distress syndrome (ARDS) often accompanies uncontrolled inflammation, oxidative processes, and injury to alveolar-capillary barrier. At present, only a few studies have used vitamin C i.v. in critically ill patients as an adjuvant therapy.

In a clinical study by Sawyer et al., high i.v. doses of **ascorbic acid and other antioxidants (**tocopherol, N-acetylcysteine, and selenium) were administered to patients with proven ARDS. This led to a **50% reduction in mortality.**

Bharara et al. administered 50 mg/kg ascorbic acid every 6 hours over a period of 96 hours for the treatment of recurring ARDS with good results and without side effects.

Fowler et al. describe the case of a 20-year-old woman with viral ARDS (rhinovirus and enterovirus D68), who was successfully treated with i.v. vitamin C. In another study patients with severe pneumonia received i.v. vitamin C. The patients treated with vitamin C showed a significantly reduced mortality rate.

Clinical research at Virginia Commonwealth University (USA) have shown that a higher plasma level of vitamin C could contain systemic infections, correct sepsis induced coagulation anomalies, and reduce vascular lesions in a pleiotropic manner (clinical study CITRIS-ALI; ID nr.: NCT02106975).

Critically ill patients often show a reduced concentration of antioxidants in circulation. Therefore, especially these patients would benefit from the treatment with vitamin C.

Additional Studies

Sawyer MAJ, Mike JJ, Chavin K (1989)
Antioxidant therapy and survival in ARDS.
Crit Care Med 17, article S153

Bharara A, Grossman C, Grinnan D, Syed A, Fischer B, DeWilde C et al. (2016)
Intravenous vitamin C administered as adjunctive therapy for recurrent acute respiratory distress syndrome. *Case Reports Crit Care* 856-871

Foweler AA, Kim C, Lepler L, Malhotra R, Debesa O, Natarajan R et al. (2017)
Intravenous vitamin as adjunctive therapy for enterovirus/rhinovirsus induced acute respiratory distress syndrome.
World J Crit Care Med 6(1):85-90

COVID-19

The first symptoms are generally high fever, chills, headache, muscle aches, and a dry cough (for additional symptoms refer to page 2, Table 2). The symptoms can develop into shortness of breath, respiratory distress, and dyspnea. The worsening of respiration is quick and requires intubation within 48 hours in many patients following the onset of symptoms.
The severity of the SARS-CoV-2 infection is reflected in the worsening of lung function. Even though the cause for this rapid worsening is not yet known, the clinical course shows similarities with macrophage activation syndrome, a secondary form of the hemophagocytic lymphohistiocytosis syndrome, in which the hyper secretion of proinflammatory cytokines damage the lung.

There is a pathological difference between „COVID-19 ARDS“ and the typical dyspnea: the extravascular lung water index (EVLWI) is normal or slightly elevated. Therefore, COVID-19 is not a typical emergency according to the definition. In addition, the elasticity of the lung is quite good, even though a severe hypoxia is present based on an intrapulmonary shunt. This suggests a microvascular and/or macrovascular illness

possibly explaining the increased D-dimer value (refer to laboratory diagnostics). Furthermore, these patients often present with a pulmonary embolism, as microthombi are often found in the pulmonary circulation at autopsy.

Laboratory diagnostics

In the early stage of the illness, white blood cells in peripheral blood are normal or decreased with a reduced lymphocyte count. Some patients show abnormal liver function and increased lactate dehydrogenase (LDH), creatine kinase (CK), and myoglobin (Mb) values. Most patients have high C-reactive protein (CRP) and globular erythrocyte sedimentation rate (ESR) values, but normal procalcitonin levels. In severe cases, an increase in D-dimer values, and possibly also other coagulation activators, can be observed. During the further progression of the illness, increased concentrations of inflammatory cytokines, such as IL-2, IL-6, and TNF-α can occur. Similarly, ferritin – the iron storage protein – increases due to the inflammation caused.

Treatment and Monitoring

Different drug treatment combinations, for example hydroxychloroquine sulfate or chloroquine phosphate with azithromycin, seem to have some effectiveness in treatment. Interferon (preferably interferon κ) is administered and various antiviral cocktails are being tested; however, their effectiveness has not been proven in clinical studies. Unfortunately, up to now, no curative treatment has been found.

In China, vitamin C i.v. is already being used in the treatment of COVID-19. At **Zhongnan Hospital at the University of Wuhan** (China), a Phase II clinical study has been registered (ID Nr: NCT04264533) to test the effectiveness: patients receive 24 g vitamin C i.v. per day over a period of 7 days. The **Shanghai Research Group for clinical treatment of COVID-19** recommends a continuous high dose in critically ill patients with SARS-CoV-2, as the continued use seems to result in a significant improvement of the oxygenation index. Several hospitals have already included administration of vitamin C i.v. in conjunction with oral zinc (220 mg) into the treatment scheme of azithromycin and hydroxychloroquine and also treat patients with minor lung involvement in the same manner. The intensive care unit of **North Shore University Hospital** (New York, USA) also is administering vitamin C at a dose of 3 g every 6 hours or 12 g per day.

Treatment protocol

To treat COVID-19-related pneumonia and hyper-inflammation, high doses of vitamin C should be given. A pro-oxidant effect of high-dose vitamin C has not yet been proven *in vivo*. The „cytokine storm" during the illness results in reactive oxygen species, which can be treated with 30–60 g vitamin C. Simultaneously, the relatively high amount of vitamin C can improve chemotaxis of white blood cells (neutrophils, macrophages, lymphocytes, B-cells, NK-cells).

Protocol for the administration of vitamin C i.v. with COVID-19 infection

A central venous access is preferred for very high doses (> 50 g)

Laboratory testing: hemogram, renal function[a], iron, ferritin, electrolytes, and glucose-6-phosphate dehydrogenase (optional)

IL-6 and especially ferritin are progression markers and markers of treatment response, vitamin C can increase the level of ferritin [b]

Use sterile water, PlasmaLyte, Ringer's lactate or physiological saline solution

Vitamin C dosing: 0.2–0.5 g/kg/d[c], , frequency of administration: 1, 2, or 4 time per day. Upon improvement of the status every 2nd day until the end of treatment.

Rate of infusion: 0.25–0.5 g/min (depending on the dose, between 1 and 4 hours)

Supplementation with calcium, magnesium, if needed[d] via enteral administration: zinc sulfate [e] 220 mg/24 h, thiamine (400 mg/d), vitamin D 5.000–10.000 IE/24 h[f], vitamin E 1600 IE/48 h, melatonin 6 mg/24 h

Protect the system for administering vitamin C from light, as vitamin C is light sensitive and easily oxidable.

[a] Care should be taken with dosing and duration of infusion, if creatinine > 175 µmol/l (1.98 mg/dl)

[b] If ferritin decreases and increases again, the dose of vitamin C should be reduced

[c] Critically ill patients can receive double daily doses (0.4–1 g/kg)

[d] The complex-forming effect of vitamin C can lead to hypocalcemia and hypomagnesemia.

[e] 220 mg zinc sulfate contains 50 mg elemental zinc.

[f] Target plasma level of 25-OH vitamin D: 80–90 nmol/l

Abb. 2: Treatment protocol and supplemental application notes according to Hernandez et al. 2020

The treatment protocol shown in Figure 2 is flexible. It will need to be adjusted according to the clinical status and the supportive treatment of the patient. For example, vitamin C dose and frequency must be adjusted in the case of additional ozone therapy (i.e. a treatment-free interval of 3 hours prior to and following the ozone treatment) as the treatments act antagonistically.

Ferritin is a good marker to evaluate the response to therapy and the prognosis. However, since vitamin C can increase the level of ferritin and may make interpretation difficult, it is necessary to use other markers, if IL-6 is not available, to adjust the dose of vitamin C.

Vitamin C and COVID-19

Conclusion

Vitamin C can be used as an effective treatment of SARS-CoV-2 due to its antioxidant and antiviral characteristics, the positive effect on the immune system, and its anti-inflammatory characteristics. In addition, vitamin C reduces the accumulation of fluids in the alveoli by preventing the activation and accumulation of neutrophils and reducing the injury to the alveolar epithelium. The administration of an i.v. infusion up to a maximum dose of 100 g/d is safe, as long as the treatment recommendations are followed (refer to figure 2).

Products for the determination of vitamin C and vitamin B_1 status:

➲ **Vitamin C**	KC2900	HPLC
	K 4000	colorimetric
➲ **Thiamine (Vitamin B_1)**	KIF001	direct MTP assay
	KC2201	HPLC

Further monitoring parameters for COVID-19:

➲ **CRP**	K 9710s	ELISA
	K 9720s	ELISA (1-point-calibration)
➲ **Iron** *(RUO)*	KJCFE005	colorimetric
➲ **Ferritin**	KD1872	ELISA

The role of Zinc in COVID-19

The trace element zinc is a co-factor, signal molecule or a structural element in numerous biological processes. It plays a role in carbohydrate and lipid metabolism and in the maintenance of reproductive, cardiovascular, and neural system functions. In the immune system, zinc has different functions in proliferation, differentiation, maturation and activity of leucocytes and lymphocytes (thymic hormone „thymulin"), and in the regulation of the immune response.

Up to 17% of people worldwide have a zinc deficiency. In particular children – specifically premature births – and older people, and also patients with diabetes, heart, and immune diseases are vulnerable. A zinc deficiency can lead to a susceptibility to inflammation and infection. Epidemiological data of populations shows that there is an increased prevalence of respiratory infections in dependence on the zinc status in children and the elderly. During the H1N1 epidemic (swine flu), zinc was suggested as a possible factor for adjuvant therapy and prevention.

The following article describes the connection between zinc deficiency and COVID-19 disease.

Publication:

Skalny AV, Rink L, Ajasuvakova OP, Aschner M, Gritsenko VA, Alekseenko SI, Svistunov AA, Petrakis D, Spandidos DA, Aaseth J, Tsatsakis A, Tinkof AA (2020)
Zinc and respiratory tract infections: Perspectives for COVID-19 (Review). *Int J Mol Med*; DOI: 10.3892/ijmm.2020.4575

Zinc has a central role in the immune system and is, therefore, considered a potential supporting substance in the therapy of COVID-19.

1. Zn^{2+} together with the zinc ionophore pyrithione inhibits SARS-Coronavirus RNA polymerase and therefore reduces the replication of the virus. Zinc therefore could be used as a virostatic agent.
2. Chloroquine, in use as a COVID-19 medication, is also a zinc ionophore and increases zinc absorption by the cells. This seems to be the anti-viral action of chloroquine. Zinc could possibly also be effective without chloroquine and its associated side effects or possibly in combination with better tolerable zinc ionophores (i.e. antioxidant polyphenols like quercetin, epigallocatechin gallate).
3. In experiments, zinc reduces ACE2 activity at physiological concentrations. The viruses could then infect cells less successfully.
4. Zinc supplementation improves the structures of cilia in the bronchial epithelium of rats with zinc deficiency. This allows the body to reduce bacterial co-infections.
5. Zinc has anti-oxidative properties and could support the anti-oxidative effects of vitamin C.
6. Zinc regulates proteins that support the function of the epithelial barrier (ZO-1 and claudin-1) and thereby inhibits uncontrolled protein loss and lung edema.

These effects are known from other virus infections and could possibly be carried over to COVID-19. . A dose of > 75 mg/d seems to significantly reduce the length of an infection. The most effective combination seems to be zinc acetate.

The target level of zinc for prevention of pneumonia is > 70 µg/dl (10.8 µmol/l). Patients whose zinc concentrations were higher than this value had a shorter time of illness. In addition, these patients needed less antibiotics compared to those with a low zinc level.

Zinc and risk groups

In particular with increasing age, and also diabetes mellitus, overweight, atherosclerosis, and immunodeficiency, zinc deficiency is increasingly present. These are the patients that belong to the risk groups where a severe course of COVID-19 can be expected.
A summary of the protective mechanisms of zinc is shown in Figure 3.

Zinc and COVID-19

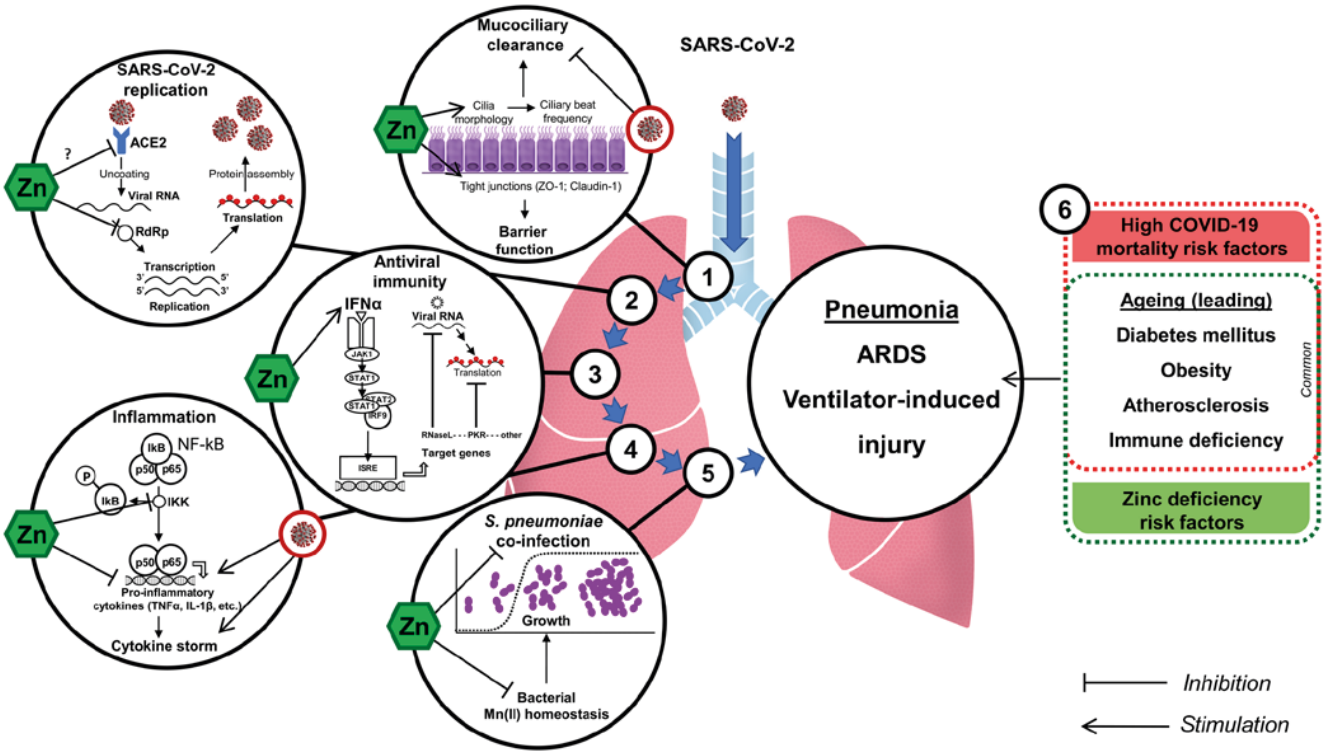


Figure 3: **Hypothetical protective characteristics of zinc for COVID-19** (Source: Skalny et al.; 2020)

1. Zinc improves cilia and their movement and thereby supports the physical defense toward microorganisms. The improvement in the *tight junctions* inhibits entry of germs.
2. Zinc prevents replication of the virus by inhibiting the RdRP gene. In addition, it is postulated that it reduces ACE2 activity and therefore further reduces replication.
3. Zinc increases production of IFN-α and thereby decreases virus replication.
4. Zinc, due to its anti-inflammatory characteristics, reduces the „cytokine storm".
5. Zinc inhibits co-infections.
6. Zinc supplementation has a positive effect for persons in the risk groups.

Outlook

There are indirect indications for the protective and antiviral effect of zinc in the control of COVID-19. Further clinical studies should be done to understand the role of zinc deficiency and the susceptibility to COVID-19 and the effects of supplementation with treatment.

Products for the determination of Zinc and Thymulin

➲ **Zinc** *(RUO)*	KJCZN001	colorimetric
➲ **Thymulin** *(RUO)*	KR9810	ELISA

Zinc deficiency

Zinc deficiency leads to an over-production of pro-inflammatory mediators. In addition, there is thymic atrophy, a reduction of naïve B-cells, an imbalance between type 1 and type 2 T-helper cells as well as an increase of type 17 T-helper cells. On the contrary, the number of regulatory T-cells decreases.

Zinc deficiency and its effects on the immune system are:

- Decreased immune resistance, increased susceptibility to infection
- Reduction of mucosa thickness, especially in the lung (bronchial tract)
- Decrease in the level of thymulin, disturbed maturation of T-lymphocytes
- Decrease in T-helper, T-killer ,and NK-cells

Gröber U, Kisters K, Schmiedel V, Friedrichsen HP, Holzhauer P, Holick MF, Classen HG, Kolisek M (2020)
Corona, Influenza & Co. – Stellenwert von Nährstoffen bei virusbedingten Atemwegserkrankungen.
Orthomol Med 18: 6-12

How long do patients remain infectious?

The following study was carried out due to the urgent need to recognize replication, immunity, and infectivity .

Publication:

Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehrmann R, Zwirglmaier K, Drosten C, Wendtner C (2020)
Virological assessment of hospitalized patients with COVID-19.
Nature Apr 1. doi: 10.1038/s41586-020-2196-x.

Nine patients with COVID-19, who had had close contact with an index patient, were studied in this investigation. All people were treated in the same hospital.

Co-infections were excluded with RT-PCR. An initial oro- or nasopharyngeal swab was taken for diagnosis. Both of the samples were used in the further course. No differences in the viral load could be determined in both materials.

Samples from day 1–5 (mild or prodromal symptoms) were positive with a mean viral load of $6.76*10^5$ copies up to day 5. After day 5 the mean viral load of the samples was $3.44*10^5$ RNA copies. The last positive probe was taken at day 28. Blood and urine showed no viral load. Individual courses of illness were rather mild (refer to Figure 4).

Seroconversion occurred on day 7 in half of the patients and by day 14 in all patients. All treated patients developed neutralizing antibodies; however, the titers did not correlate with the clinical course. Patient Nr 4. had the lowest titer of neutralizing antibodies and excreted viruses via stool. Cross-reactivity of antibodies with four endemic coronaviruses was confirmed in some patients.

PATIENT ID	COMORBIDITY	INITIAL SYMPTOMS	LATER SYMPTOMS	ANC/µl	ALC/µl	CRP (mg/l)	LDH (U/l)
#1	hypothyreoidism	cough, fever, diarrhea	diarrhoea	4870	1900	46	197
#2	none	sinusitis, cephalgia, cough,	hyposmia, ageusia	3040	1200	4.9	182
#3	COPD	arthralgia, sinusitis, cough,	dysosmia, dgeusia	5040	2600	1.3	191
#4	none	otitis, rhinitis,	hyposmia, hypogeusia	2420	2220	5.9	149
#7	hyper-cholesterinemia	rhinitis, cough,	fever, dyspnea, hyposmia, hypogeusia	4690	900	4.9	209
#8	none	sinusitis, cough		2500	1600	1.7	203
#10	none	sinusitis, cough,	fever, cough	2350	700	7.8	220

Abbreviations: ANC = absolute neutrophil count, ALC = absolute lymphocyte count, CRP = C-reactive protein, LDH = lactate dehydrogenase, M = male, F= female

Figure 4: Courses of illness according to Wölfel et al.; 2020

The correct confirmation of SARS-CoV-2

RT-PCR

RT-PCR is the gold standard for the diagnosis. Initial tests were established in January 2020 as in-house procedures. In the meantime, several, even CE-certified, methods have become commercially available and can be used in automated applications.

Occasionally, recovered patients test positive for SARS-CoV-2 specific nucleic acids for a longer time period. The cause and significance in relation to the infection is not yet fully elucidated.

Proof of antibodies

The determination of antibodies is used in the confirmation of the reaction of the immune system in the fight against infection.

In the laboratory, ELISA is the test of choice. It can prove viral titers for specific antibodies of the IgM type (early infection phase, as of day 7) and IgG (late infection phase, as of day 14). The sensitivity of the individual confirmatory methods is dependent on the time point of the sampling and the course of the infection (refer to Figure 5).

Processing ELISAs requires experience and can be performed (semi)automatically.

Publication:

Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N, Zhang Z. (April 30, 2020)
Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019.
Clin Infect Dis; 2020 Mar 28. pii: ciaa344. doi: 10.1093/cid/ciaa344.

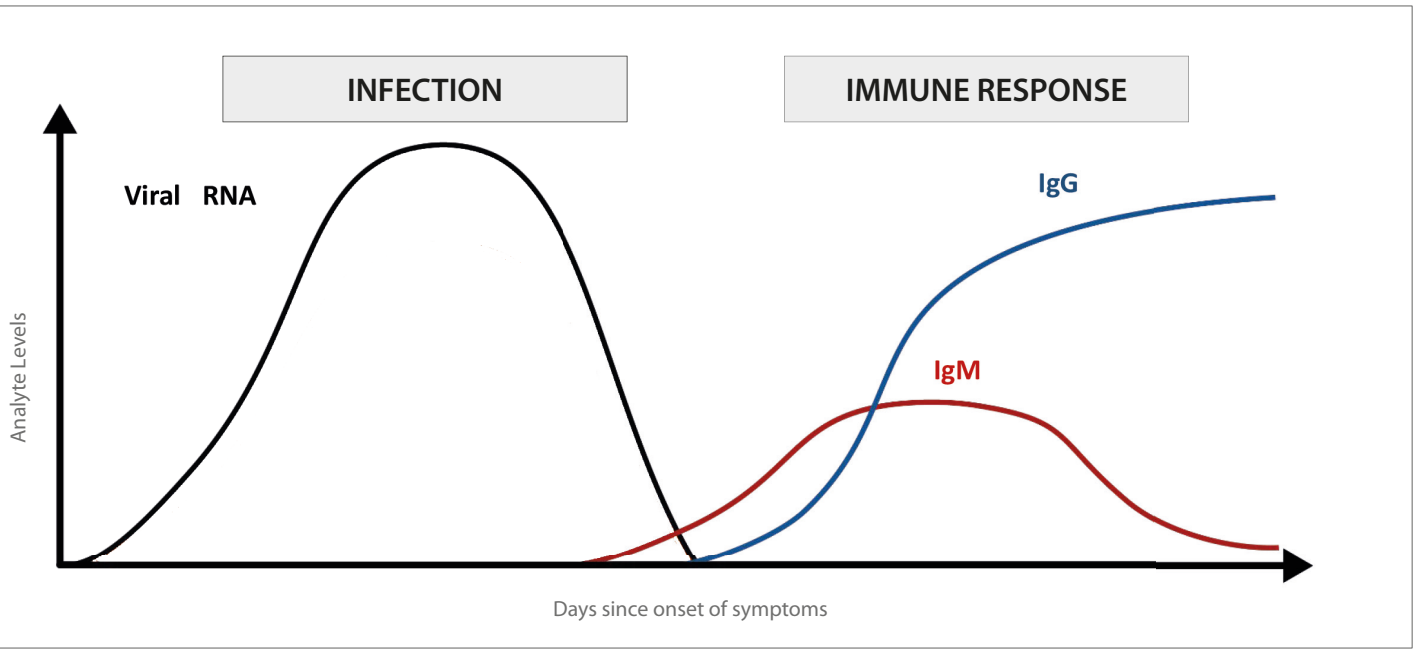


Figure 5: Course of different markers of infection with SARS-CoV-2

LABORATORY TIP

Immunofluorescence microscopy

This complex detection method offers subjective evaluations and is only applicable for specific issues.

Neutralization tests

This biological test confirms antibody presence against a specific pathogen and is highly specific. However, it is labor-intensive, requires specific laboratory equipment, and is only partially automatable.

Rapid tests

A differentiation needs to be made between the confirmation of viral antigen and confirmation of specific antibodies. Both tests can give results within a few minutes. Immunochromatographic rapid tests can be meaningful as supplemental clinical methods (PCR) and should only be processed by professional users.

Which tests are also meaningful?

Clinical Chemistry	A	I	N
Na, K, glucose	x	x	x
Creatinine	x	x	x
Urea		x	
ALT (GPT), LDH	x	x	x
CK, bilirubin (total)	x	x	
CRP	x	x	x
PCT		x	
as needed: Troponin Ferritin Albumin			

Hemogram	A	I	N
Hemogram and automated differencial blood count	x	x	x
Lymphocytes	x	x	x

Coaggulation	A	I	N
Quick	x	x	
aPTT	x	x	
as needed: D-Dimer			

Table 7: Important parameters to aid in the interpretation of COVID-19; A = at admission, I= intensiv care unit (ICU) course control, N = regular unit course control; based on „Empfehlung der Deutschen Gesellschaft für Klinische Chemie und Laboratoriumsmedizin; von Ahsen N: Brief der DGKL an die Mitglieder vom 7. April 2020" (Recommendation of the German Society for Clinical Chemistry and Laboratory Medicine; von Ahsen N: letter of DGKL to the members, April 7, 2020)

Interpretation aids

CRP – 90% of the patients have increased values during viral infections (20–80 mg/l)

PCT – in severe courses (ICU) values are >0.1 to >0.5 µg/l. This usually indicates a bacterial superinfection. At admission, most patients present with unremarkable PCT concentrations.

LDH – in severe courses (ICU) >245 U/l, for pulmonary and/or multiorgan injury >450 U/l. After >14 days the values for LDH should decrease. If this is not the case, it is predictive of an unfavorable clinical course.

Increasing **creatinine** and **urea** after 10 days of treatment predict an unfavorable further course of the illness.

Increased **total bilirubin** and **ALT (GPT)** are indications of concomitant hepatic injury.

Troponin T is increased in severe courses (ICU) and especially in virally induced cardiac injury (>14 ng/l). Early increasing values for troponin T appear often in unfavorable courses and continue to increase in the 1st and 2nd week of treatment.

LABORATORY TIP

Ferritin: in severe courses and questions concerning ARDS, excessive levels of ferritin of 1000 up to 10,000 µg/l are observed.

The **hemogram** often shows leucopenia, leukocytosis, neutrophilia, and lymphopenia or alternatively eosinopenia.

D-dimer increases in the initial days of illness are an unfavorable prognostic indication. In severe cases, concentrations of >500 µg/l can occur.

A decrease in the **Quick PT % value** is also an unfavorable indication.

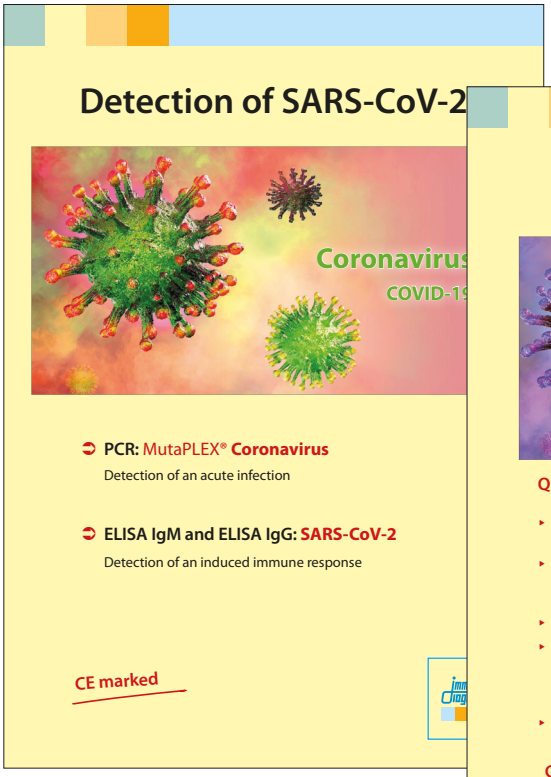
In severe courses, nephritis and *Capillary Leak Syndrome* can occur. Albumin is excreted in the urine and needs to be monitored by laboratory diagnostics.

Products for COVID-19 diagnostics:

➲ MutaPLEX® **Coronavirus**	KG192696	qualitative Real-Time-RT-PCR (96 tests)
	KG1926-384	qualitative Real-Time-RT-PCR (384 tests)
	KG1926-768	qualitative Real-Time-RT-PCR (768 tests)
➲ **Coronavirus COVID-19 IgM**	KKT1033	ELISA
➲ **Coronavirus COVID-19 IgG**	KKT1032	ELISA

Further monitoring parameters for COVID-19:

➲ **CRP**	K 9710s	ELISA
	K 9720s	ELISA (1-point-calibration)
➲ **Ferritin**	KD1872	ELISA
➲ **Albumin**	K 6330	ELISA



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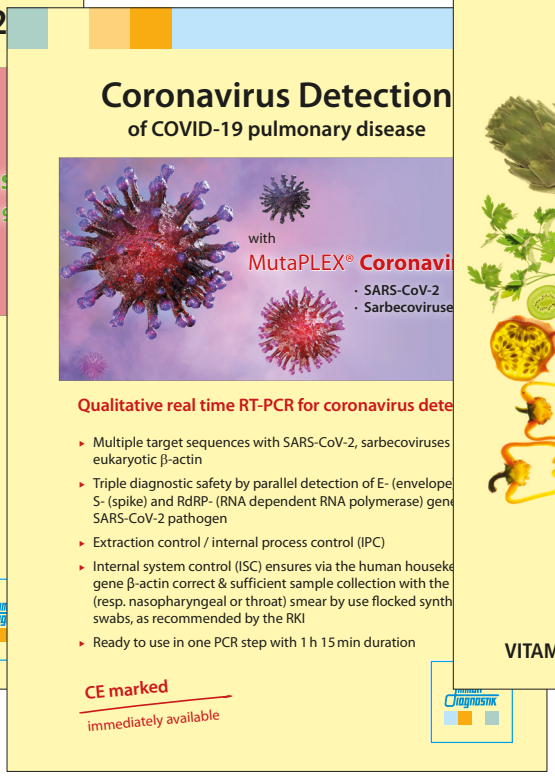

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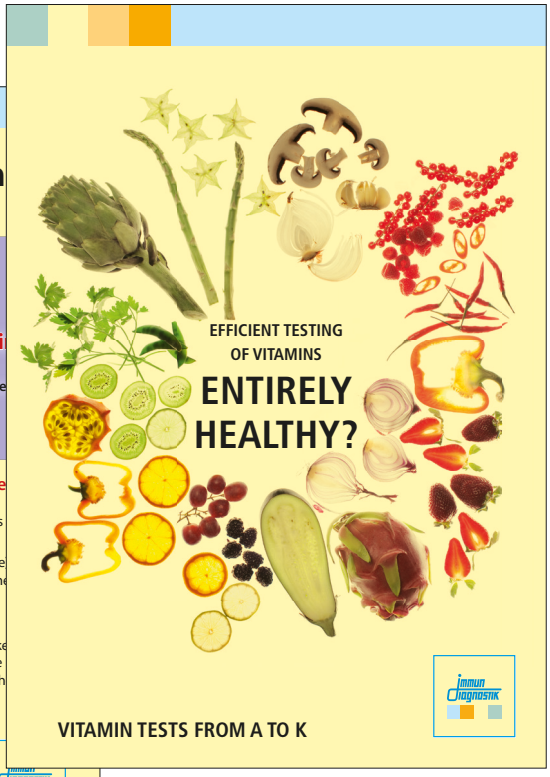

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