Donor Selection Guideline

SARS-CoV-2 / COVID-19

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Conclusions

Must not donate if

- The donor has an active infection with SARS-CoV-2
- There is a suspicion of an infection with SARS-CoV-2 at the time of death
- The donor has recovered from an infection with SARS-CoV-2 but has been symptom-free for less than 28 days
- The donor has been in close contact with a person with a confirmed SARS-CoV-2 infection in the past 28 day

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Disclaimer

The information in this section was written based on the current knowledge about SARS-CoV-2. Although new publications are added on a daily basis, the knowledge about this virus is still fairly limited. This means that the conclusions in this document are preliminary conclusions that may change in the future.

Summary of characteristics

COVID-19 disease presentation	
Incubation time	Average 5 to 6 days, distribution of 2 to 14 days
Symptoms	*Mild* • Runny nose • Sore throat • (dry) Cough • Fatigue • Increased sputum production • Muscle and joint pain • Headache • Elevated temperature • Also reported (in a smaller portion of the patients): diarrhoea (4%); nausea and vomiting (5%). In addition, the loss of sense of smell (hyposmia/anosmia) and taste (dysgeusia) have also been reported. *Severe* • Fever (>38 degrees Celsius), • Shortness of breath • Pneumonia • Acute respiratory stress syndrome • Septic shock
Diagnostic tools	*Direct:* • Detection of viral RNA by means of (real-time) reverse transcription (RT)-PCR in samples obtained from nasal or oral swabs *Indirect:* • Serology/CT scan/Lab values (increased CRP, absolute lymphocyte count < 1500 µ/L)
Transmission	*Direct:* • Transmission via droplets that are emitted by coughing, sneezing, talking and breathing, similar to influenza. Droplets generally do not travel more than 2 metres. • Via aerosols that are released during aerosol-forming procedures (tracheal intubation, non-invasive ventilation, tracheostomy, cardiopulmonary resuscitation, manual ventilation prior to intubation, bronchoscopy, procedures involving the tracheostomy, suctioning). *Indirect:* • Infection could also occur if an individual touches an infected surface and then touches his or her eyes, nose or mouth. *N.B. Transmission of the virus by asymptomatic individuals has been described in various articles, but it is not certain to what extent this occurs.*
Risk factors	• Abnormalities and functional impairment of the airways and lungs; • Chronic heart conditions; • Diabetes mellitus; • Chronic kidney conditions requiring dialysis or kidney transplant; • Reduced immunity to infections caused by medication for auto-immune disease, following organ transplant, due to haematological conditions, due to congenital conditions or conditions acquired at a later stage in life. • Obesity
Immunity	Seroconversion after approximately 10 days. Not yet clear for how long the immunity remains.

Can SARS-CoV-2 be transmitted via tissue transplants?

Preliminary conclusions

The potential risk of transmission of SARS-CoV-2 has been set out below per tissue type. Although transmission of the virus via tissue transplantation seems unlikely, a potential infection with SARS-CoV-2 can have very serious consequences. SARS-CoV-2 is a pandemic virus with a high mortality and morbidity to which people have barely developed immunity. Our knowledge about the virus is currently still limited. Therefore, the NTS currently deems the standard and additional screening of postmortem tissue donors essential, including RT-PCR test of nose/throat swabs.

Donor testing

The NTS has commissioned Sanquin to test swabs collected from the nose and throat of postmortem tissue donors for the presence of viral RNA. Sanquin uses the Cobas® SARS-CoV-2 real-time RT-PCR for this purpose. According to the manufacturer, a positive test result is indicative of the presence of SARS-CoV-2 RNA, but this result does not rule out a bacterial infection or a co-infection with other viruses. For the NTS, a positive result forms an immediate reason not to release the tissues. A negative result also does not rule out infection with SARS-CoV-2. Therefore, the medical staff of the NTS will always interpret a negative test result in the context of the clinical presentation at the time of death, the medical and social history of the donor and any diagnostic tests that were performed before the donor's death, such as a CT scan. The NTS is investigating whether a serological test - in addition to the RT-PCR test - has any added value for the donor screening.

Eye tissue

SARS-CoV-2 uses the ACE2 receptor for the infection of cells. Infection also depends on the protease activity of the TMPRSS2 protein. ACE2 RNA expression has been found to a very limited extent in the cornea. The same is true for the expression of TMPRSS2.[1] The receptor also appears to be present in the aqueous humor, where it may play a role in the intra-ocular RAS system.[2] As yet, there are no indications that SARS-CoV-2 can infect the cornea or sclera and thus be transmitted to the recipients. A SARS-CoV-2 infection appears to be associated with the development of conjunctivitis in a limited number of cases. Viral RNA also occasionally appears to be present in the lachrymal fluid, as was demonstrated for SARS-CoV in 2004. However, reports about this are contradictory. A recent study by Jun *et al* found no infectious virus or viral RNA in the lachrymal fluid of 17 COVID-19 positive patients.[3,4]

Processing of eye tissue

The literature appears to indicate that the treatment of eye tissue with ethanol and biocides - such as povidone-iodine - effectively inactivates different viruses with and without a membrane (envelope), including the MERS-coronavirus.[5] The study into the inactivation of MERS-CoV used three antiseptic products under the brand name Betadine, at concentrations ranging from 1% to 7.5%. According to the authors, the 4-log reduction that was

achieved in all cases equates to an inactivation of 99.99%. The eyeball is rinsed with povidone-iodine during extraction (both on site upon extraction from the donor and upon arrival at the tissue bank). In addition, according to ETB-BISLIFE, scleral tissue is physically cleaned and then stored in high-grade pure alcohol for preservation and disinfection. On average, the scleral tissue remains in the alcohol for 6 to 11 months before being released. The alcohol is also replaced by new alcohol during this period. These processing steps further reduce the risk of transmission of any virus particles that are present.

Skin

A study by Hamming *et al.* from 2004 reveals that the ACE2 receptor is present in the basal cell layer of the epidermis and in the smooth muscle cells surrounding the sebaceous glands.[6] It is not known whether the virus actually infects the skin or can infect the skin. The virus can end up on the skin (such as the hands) via coughing and sneezing and can remain infectious there for a short period. It is not known how long this period lasts, but for Influenza – also an enveloped respiratory virus - this period is approximately 10 minutes. This would mean that there is a risk of transmission of the virus during the harvesting of the skin. However, a donor is no longer breathing and therefore no longer actively shedding the virus via the respiratory tract. In addition, the skin is thoroughly disinfected and processed (see below).

Processing of skin tissue

Before the skin is harvested, the donor is first washed with Betadine scrub (polyvinylpyrrolidone-iodine complex). According to ETB-BISLIFE, this liquid is then left on the donor's skin for 10 minutes to ensure optimum disinfection. The skin then undergoes further disinfection with chlorhexidine/alcohol solution. The harvested skin is preserved in glycerol for an extended period. The literature states that various types of viruses are inactivated or eliminated during the storage (> 4 weeks) in glycerol at a temperature of at least 20 °C.[7] It is not known to what extent this also applies to coronaviruses, but the combined action of physical cleaning, disinfection with Betadine and chlorhexidine/alcohol and the further virucidal effects of glycerol probably make the risk of transmission of any virus particles that are present negligibly low.

Heart valves

Although the clinical manifestations of COVID-19 are dominated by the respiratory symptoms, some patients also develop fulminant myocarditis or other cardiac problems.[8,9] These complications are probably the result of an excessive immune response to the virus and localised vascular inflammation. There is currently no evidence supporting the presence of infectious virus in the heart, but this also cannot be ruled out due to the presence of the ACE2 receptor in the heart.[10] An indication for the potential presence of the virus in the heart is provided by an autopsy study of the first SARS virus in 2003 (This virus binds to the same receptor as the new SARS virus). In this study, viral RNA was detected in 35% (7/20) of the heart samples obtained from deceased SARS patients in Toronto.[11] In a recent autopsy study of 12 patients who died of COVID-19, a small amount of viral RNA of SARS-CoV-2 was detected in the heart tissue of 1 person who died with myocarditis.[12] If SARS-CoV-2 can actually infect the heart muscle cells, then this points to a systemic viral infection. Such a systemic infection is a contraindication for tissue donation.

Processing of heart valves

According to ETB-BISLIFE, heart valves do not undergo any specific virus-reducing treatment. However, the harvested heart is stored at the tissue bank in Ringer's irrigation solution until further processing and the prepared tissues are stored in preservation medium. As these liquids rinse the tissue, they probably contribute to some reduction of any virus present around the tissue, though this effect is probably limited. In the case of vascular and heart valve tissue, careful screening of the donor with regard to recent infectious respiratory conditions in combination with elevated systemic infectious parameters will need to be used to provide clarity about any risks of transmission. This will need to be evaluated on a case-by-case basis. According to ETB-BISLIFE, the remaining heart tissue from each donor undergoes histopathological analysis after dissection. This makes it possible to detect any local inflammation in the heart tissue, such as COVID-19-related myocarditis.

Bone and tendon tissue

As yet, there is no scientific evidence that SARS-CoV-2 can infect bone and muscle tissue. To the best of our knowledge, the ACE2 receptor is not present in bone tissue. The receptor has also not been detected in bone marrow. The donor will not be accepted if there are any signs of a systemic infection.

Processing of bone and tendon tissue

ETB-BISLIFE sends all harvested bone/tendon tissue from postmortem donors for its own tissue bank for processing by BST (Barcelona, Spain) or supplies these tissues to DIZG (Berlin, Germany) for processing and distribution elsewhere by DIZG. The tissues that are processed by BST undergo stringent physical and chemical cleaning (irrigation with water and detergents, centrifugation, ultrasonic treatment) and disinfection (hydrogen peroxide and alcohol). In addition to cleaning and disinfection, tissues supplied by ETB-BISLIFE to DIZG also undergo sterilisation (peracetic acid).

The cleaning, disinfection and sterilisation procedures performed by BST and DIZG are validated. Based on these validation studies, it is assumed that the processing method is effective for the inactivation of different enveloped viruses, including HIV, HTLV, HCV, HBV, CMV, Herpes, Rubella, Parainfluenza, Mumps, Vaccinia, Influenza. ETB-BISLIFE states that an 8-10 log reduction can be achieved for these viruses. Although SARS-CoV-2, SARS-CoV and MERS-CoV have not been included in the aforementioned validation studies, it is probable - based on their similar structure - that an effective reduction and inactivation of SARS-CoV-2 can be expected during the processing of bone and tendon tissue. Based on this information, ETB-BISLIFE deems the risk of the presence of coronavirus in processed bone/tendon tissue to be negligibly low.

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory illness that is caused by a novel coronavirus called SARS-CoV-2 (*Severe Acute Respiratory Syndrome Coronavirus 2*). The whole genome sequences from five patient isolates were published soon after the discovery of the virus. This revealed that the new virus belongs to the genus *Betacoronavirus*, family *Coronaviridae*. [13]

SARS-CoV-2 is the third highly pathogenic coronavirus that has jumped from animals to humans in the past 20 years. The two other viruses, called SARS-CoV (8,096 cases/774 deaths) and MERS-CoV (2,494 cases/858 deaths) caused a global epidemic in 2003 and 2012 respectively (the genome sequence of SARS-CoV-2 exhibits 82% similarity to that of SARS-CoV). The SARS epidemic started in China and spread to other parts of the world via travellers. The MERS epidemic started in Saudi Arabia and also spread to other parts of the world via travellers. A major difference compared to the current coronavirus is that SARS-CoV and MERS-CoV are much more deadly and only people who were symptomatic were able to spread the virus. The main route of transmission was nosocomial.[14] In contrast, SARS-CoV-2 causes only mild symptoms in many cases, which are similar to a common cold. This means that the virus can spread much more easily amongst the general population, via people with mild symptoms.

In addition to the high pathogenic coronaviruses described above, there are also 4 low pathogenic coronaviruses circulating in the population, namely HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1. These viruses cause a common cold. Most people will become infected with one or more of these viruses at some point in their lives.[15]

Incubation time

Average 5 to 6 days, distribution of 2 to 14 days.[23]

Symptoms

Mild symptoms: Runny nose (catarrh), sore throat, (dry) cough, fatigue, increased sputum production, muscle and joint pain, headache, elevated temperature (<38 degrees Celcius).

Severe symptoms: Fever (>38 degrees Celsius), shortness of breath, pneumonia, acute respiratory distress syndrome, septic shock.

Diarrhoea, nausea and vomiting have also been reported in some patients. In addition, the loss of sense of smell (hyposmia/anosmia) and taste (dysgeusia) have also been reported. The LCI guideline on COVID-19 describes a retrospective study of 214 COVID-19 patients in Wuhan, China. Neurological symptoms were reported in 36.8% of the patients. Of these patients, 24.8% had symptoms affecting the central nervous system, such as headache, dizziness, ataxia, epilepsy and acute cerebrovascular disease. A further 8.9% had peripheral neural conditions, including 5% hyposmia. Hypogeusia, hypogia and neuralgia were also reported.[23]

Pathogenesis and tropism

Coronaviruses are characterised by the presence of protrusions that project from the cell membrane in the shape of a crown (corona). These so-called Spike proteins (S) are responsible for the binding of the virus to the host cell and the fusion of the viral membrane with the cell membrane of the host cell. This two-step process is facilitated by the different sub-units of the S protein, called S1 (for binding) and S2 (for fusion). During this process, the viral RNA is released in the cytoplasm of the host cell and the infection has taken place. Research has demonstrated that the S protein of SARS-CoV-2 binds to the *angiotensin-converting enzyme 2* (ACE2) receptor. The protease TMPRSS2 is needed to cleave the S protein into the S1 and S2 subunit.[16]

This receptor is also used by the previously described SARS-CoV (MERS uses a different receptor called dipeptidyl peptidase 4 (DPP4)). The major difference is that the binding with SARS-CoV-2 is probably stronger and more efficient than with SARS-CoV. This can mean, for example, that fewer virus particles are required to cause a more effective infection. Another difference is that the S protein of the new SARS virus has a so-called furin binding site, which is cleaved by the enzyme furin. It is not entirely clear what this all means for the virus

The ACE2 receptor is normally expressed at different sites in the body, including type II alveolar cells in the lung, the upper and stratified epithelial cells in the oesophagus, in the blood vessels, on enterocytes of the ileum and colon, on cholangiocytes in the bile duct, on myocardial cells, on proximal tubular kidney cells and on bladder urothelial cells. All these organs and tissues can potentially be infected by the virus.[17] This was confirmed by a recently published autopsy study by Bradley *et al.* In this study involving 12 deceased individuals, virus particles were detected in organs including the lungs, the trachea, the kidneys and the intestines. All the deceased had significant co-morbidity, such as hypertension, obesity, chronic kidney conditions and diabetes.[12]

Recent studies on the expression of ACE2 and TMPRSS2 show that of all the cells tested in the respiratory tract, the epithelial cells in the nose (in particular, ciliate cells and goblet cells) exhibit the highest expression of ACE2 and TMPRSS2 mRNA. This could explain why the virus can spread more efficiently between people compared to, for example, the MERS coronavirus, whose receptors are mainly in the lower respiratory tract.[1]

As a result of the infection, the expression of the ACE2 receptor is significantly reduced (down-regulated). ACE2 normally plays a role in reducing blood pressure through the production of a vasodilator called Ang1-7 in the renin-angiotensin system. Loss of ACE2 expression in the lungs is associated with pulmonary hypertension, sarcoidosis, idiopathic pulmonary fibrosis and acute respiratory distress syndrome (ARDS).[18] The immune system is also activated, which in severe cases can result in the uncontrolled release of cytokines (cytokine release syndrome or CRS). This too can cause ARDS and in severe cases can result in multi-organ failure and fulminant myocarditis.[19,20,21] Multi-organ failure in COVID-19 patients may be the result of the synergistic effects of tissue damage induced by SARS-CoV-2 and a systemic cytokine storm. However, this still needs to be studied in more detail.[22]

Transmission

Transmission takes place via droplets that are emitted by coughing, sneezing, talking and breathing. The droplets generally do not travel more than 2 metres. Transmission can also take place via aerosols that are released during aerosol-forming procedures (tracheal intubation, non-invasive ventilation, tracheostomy, cardiopulmonary resuscitation, manual ventilation prior to intubation, bronchoscopy, procedures involving the tracheostomy, suctioning). Infection could also occur if an individual touches an infected surface and then touches his or her eyes, nose or mouth.[23]

A recent cohort study and a meta-analysis of published articles reveal that the pooled prevalence of gastro-intestinal symptoms (including nausea, vomiting, diarrhoea and abdominal pain) in COVID-19 patients is 17.6%. Viral SARS-CoV-2 RNA was detected in the faeces of 48.1% of the patients. In many cases the RNA is detected even after the respiratory samples proved negative. Viral RNA was detected more frequently in patients with diarrhoea than in patients without diarrhoea.[24] This appears to suggest that faecal viral shedding is a potential route of transmission. More research is required into the extent of transmission via the faecal-oral route.[25]

Infectious period

A lot remains unclear about the extent to which the virus is shed by patients and for what period of time a patient remains infectious. In addition, a lot still remains unclear about the extent to which asymptomatic patients are infectious.

The amount of virus particles provides a measure of the contagiousness of the virus. The higher the viral load, the more contagious the disease. SARS-CoV-2 reaches peak concentration within 5 days after the start of the symptoms and the concentration is approximately 1000 times higher than the first SARS virus (SARS-CoV reaches peak concentration approximately 7-10 days after the start of the symptoms).[25] The viral load slowly decreases after the first week. Older patients and patients with co-morbidity - such as diabetes mellitus, hypertension and other cardiovascular conditions - probably have a higher viral load and probably shed the virus for a longer period.[26] In a study by To *et al*, viral RNA was detected in posterior oropharyngeal saliva samples from 7 of the 21 patients more than 20 days after the start of symptoms. According to the authors, there was no association between the severity of the disease and the prolonged detection of viral RNA. However, they also saw no difference in the initial and peak viral load between patients with and without co-morbidity.[27]

Cases of asymptomatic COVID-19 infections have been reported. Although much remains unclear about the proportion of these asymptomatic infections among all infected individuals, one Chinese study suggests that the majority of infections are asymptomatic. Pre-symptomatic transmission of the virus can occur 1 to 3 days before the development of symptoms.[28]

Immunity

In the previously mentioned study by To *et al.,* the concentration of IgG and IgM antibodies started to increase approximately 10 days after the start of symptoms. In most of the patients in this study, seroconversion occurred within the first 3 weeks after the start of symptoms. This is comparable to the results of a study by the Erasmus MC (seroconversion after 13-21 days).[29] It is not yet clear for how long after infection the immunity remains. Sanquin intends to study whether the antibodies of recovered patients can help to reduce the symptoms in other patients. They have started the collection of blood plasma from recovered COVID-19 patients for this purpose.[30]

Survival outside the host and inactivation

The stability of the virus has been studied in the laboratory. This research examined both the stability in droplets and the stability on various surfaces, such as plastic, stainless steel, copper and cardboard. The study revealed that the virus can remain viable in the air for several hours and on plastic and stainless steel for several days.[31] It should be noted here that the experiments were performed in a controlled environment and the results may differ outside the laboratory. These experiments also used high concentrations of the virus and it is not certain whether infected patients can contaminate surfaces to such an extent. According to a study by Kampf *et al.*, disinfection of surfaces with sodium hypochlorite or 62-71% ethanol results in a significant reduction of the contagiousness of coronaviruses.[32]

Diagnostic tools

Molecular diagnostics using RT-PCR

The gold standard for detection of a SARS-CoV-2 infection is (real-time) reverse transcription (RT)-PCR on isolated viral RNA obtained from samples that have been collected via nasal or oral swabs. The literature has since demonstrated that this does not offer a 100% guarantee. Yang *et al.* examined the accuracy of the various sampling techniques that are used for the laboratory diagnosis of SARS-CoV-2.[33]

Their research, based on 866 samples obtained from 213 patients (205 throat swabs, 490 nose swabs, 142 sputum samples, 29 BAL fluid samples), revealed that sputum samples are probably the most accurate, followed by nose and throat swabs. These are also the fastest, simplest and safest ways to collect samples. The authors also indicated that CT scans can form an important additional diagnostic tool for the diagnosis of *Novel Coronavirus Pneumonia* (NCP).

In a study by Wikramaratna *et al.,* 298 test results from 30 positive patients (150 nasal swabs and 148 throat swabs) were analysed and used for modelling. According to the models in the study, the chance of a positive test result decreases as the number of days since the start of symptoms increases. For a nasal swab, the percentage chance of a positive test decreases from 94.39% on day 0 (distribution 86.88% - 97.73%) to 67.15% on day 10 (distribution 53.05% - 78.85%) and 2.38% on day 31 (distribution 0.60% - 9.13%). The percentages are

slightly lower for a throat swab: 88% on day 0 (distribution 75.18% - 94.62%), 47.11% on day 10 (distribution 32.91% - 61.64%) and 1.05% on day 31 (distribution 0.24% - 4.44%).

The authors concluded that the testing of nose and throat swabs by RT-PCR does not guarantee a positive test result. The chance of a positive test result decreases as the number of days since the start of symptoms increases. In other words, the longer the time from the start of the symptoms, the higher the risk of a false-negative result. Repeated testing of suspected patients with RT-PCR negative results reduces the risk of infected individuals not being identified.[34]

The NTS has commissioned Sanquin to test for the presence of viral RNA in samples (swabs) collected from the nose and throat of postmortem tissue donors. Sanquin uses the Cobas® SARS-CoV-2 real-time RT-PCR for this purpose. According to the manufacturer, a positive test result is indicative of the presence of SARS-CoV-2 RNA, but this result does not rule out a bacterial infection or a co-infection with other viruses. For the NTS, a positive result forms an immediate reason not to release the tissues. A negative result also does not rule out infection with SARS-CoV-2. Therefore, the medical staff of the NTS will always interpret a negative test result in the context of the clinical presentation at the time of death, the medical and social history of the donor and any diagnostic tests that were performed before the donor's death, such as a CT scan.

Serological tests for SARS-CoV-2

In April, Sanquin will start measuring antibodies against the coronavirus in all blood donors who come to donate blood over a period of approximately one week.[35] The aim is to gauge how quickly the population is acquiring immunity to the disease. This can also provide insight into determining the disease burden, particularly the number of asymptomatic infections and to provide a more accurate estimate of morbidity and mortality. Sanquin will use the so-called SARS-CoV-2 Antibody Elisa for this. This assay detects the total number of antibodies against the receptor binding domain of the SARS-CoV-2 spike protein. The assay does not distinguish between IgG and IgM antibodies. The NTS is investigating whether a serological test - in addition to the RT-PCR test - has any added value for the donor screening.

Laboratory findings

Hospitalized COVID patients often appear to have an abnormal blood count. Lymphopenia and elevated CRP are particularly common. From a description of the characteristics of 107 patients with COVID-19 at the Emergency Room (SEH) of Bernhoven hospital in The Netherlands, 51% of the patients had a CRP ≥ 50 mg/l, 12% had a leucocytosis and 61% had an increased LD value.[36] The number of lymphocytes was determined in 31 patients. Absolute lymphocytopenia was present in 77% of these patients. In a series of 393 COVID-19 patients in New York City, 90% had a lymphocyte count of < 1,500/microL. Leukocytosis (> 10,000/microL) and leukopenia (<4,000 / microL) were reported in approximately 15 percent of patients.[37]

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