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*To my dear wife Sofia, and our beloved children Fiona and Topias.*

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## ABSTRACT

**Background**: The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), led to global actions to limit the transmission. Extensive testing quickly led to a worldwide shortage of testing equipment, including nasopharyngeal swabs (NPS), warranting novel specimen collection methods. Social restrictions were implemented on children, although the children's role in the household transmission of respiratory infections, both negative and positive for SARS-CoV-2, is lacking. This thesis was conducted to investigate novel specimen collection methods and household transmission of SARS-CoV-2.

**Methods:** We assessed the performance of saliva and Finswab, NPS made via plastic injection molding, compared to a reference NPS for SARS-CoV-2 detection. Household transmission of SARS-CoV-2 was examined by prospectively following acute respiratory tract infections in 700 participants in 175 households with school-aged children. Symptomatic and exposed participants were tested by SARS-CoV-2 PCR, and SARS-CoV-2 antibodies were determined five weeks after SARS-CoV-2 infection in household. Secondary attack rates were calculated.

**Results:** The number of virus-positive cases for Finswab and reference swab, and for saliva and NPS, were similar. SARS-CoV-2 $C_T$ values for saliva were higher compared to NPS. Most (90%) SARS-CoV-2 infections occurred from January to April 2022, when Omicron BA.1 and BA.2 were dominant variants. SARS-CoV-2–positive infections were transmitted more often than other respiratory infections, i.e. SARS-CoV-2–negative infections. Transmission of SARS-CoV-2–negative infections, but not that of SARS-CoV-2–positive infections, was higher for child index cases.

**Conclusions:** Finswab and saliva are both viable alternatives to the routinely used flocked nylon NPS in SARS-CoV-2 diagnostics. The household transmission of SARS-CoV-2 Omicron variants was higher compared to other respiratory infections, with children being more infective than adults only for SARS-CoV-2-negative infections.

KEYWORDS: COVID-19, household transmission, nasopharyngeal swab, saliva, SARS-CoV-2, serology

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## TIIVISTELMÄ

**Tausta:** SARS-CoV-2 viruksen aiheuttama COVID-19 pandemia johti maailmanlaajuisiin rajoitustoimiin. Näytteenoton räjähdysmäisen kasvun seurauksena näytemateriaaleista kuten nenänielutikuista oli pulaa, ja uusia näytteenottomenetelmiä tarvittiin lisää. Lapsiin kohdistettiin rajoitustoimia, vaikka lasten roolista SARS-CoV-2-positiivisten tai -negatiivisten infektioiden leviämisessä on vain rajallisesti tietoa. Tässä väitöskirjassa tutkittiin SARS-CoV-2 viruksen leviämistä perheissä sekä uusia näytteenottomenetelmiä.

**Metodit:** Väitöskirjassa tutkittiin sekä sylkinäytteen että ruiskuvalutekniikalla valmistetun nenänielutikun, Finswabin, toimintaa virusdiagnostiikassa perinteiseen nenänielutikkuun verrattuna. SARS-CoV-2 viruksen leviämistä perheiden sisällä tutkittiin seuraamalla 175 lapsiperheen ja 700 osallistujan hengitystieinfektioita. Seuranta-aikana SARS-CoV-2 oireisilta otettiin PCR-näyte ja SARS-CoV-2-positiivisen perheenjäsenet testattiin kahdesti PCR-näytteellä. SARS-CoV-2 vasta-aineet määritettiin 5 viikon kuluttua positiivisesta infektiosta.

**Tulokset:** Sekä Finswabilla ja verrokkitikulla että sylkinäytteellä ja nenänielutikulla havaittiin samankaltaiset määrät virusinfektioita. Sylkinäytteen $C_T$-arvot SARS-CoV-2 virukselle olivat nenänielutikkua korkeammat. Yli 90 % SARS-CoV-2-infektioista ajoittui tammi-huhtikuuhun 2022, jolloin Omicron BA.1. ja BA.2. olivat hallitsevat virusvariantit. SARS-CoV-2 infektiot levisivät SARS-CoV-2-negatiivisia infektioita herkemmin. Lapset levittivät SARS-CoV-2-negatiivisia infektioita aikuisia tehokkaammin, mutta tartuttavuus SARS-CoV-2 infektioissa oli yhtäläistä.

**Johtopäätökset:** Finswab ja sylkinäyte ovat molemmat potentiaalisia vaihtoehtoja perinteiselle nenänielutikulle. SARS-CoV-2 Omicron virus tarttuu perheissä herkemmin kuin SARS-CoV-2-negatiiviset hengitystieinfektiot. Lasten tartuttavuus oli aikuisia suurempaa ainoastaan SARS-CoV-2-negatiivisissa infektioissa.

AVAINSANAT: COVID-19, koronavirus, nenänielunäyte, perhetutkimus, SARS-CoV-2, sylkinäyte, tarttuminen, vasta-aineet

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# Abbreviations

| | |
|---|---|
| 3D | Three-dimensional |
| ACE2 | Angiotensin-converting enzyme 2 |
| AI | Artificial intelligence |
| ARDS | Acute respiratory distress syndrome |
| cDNA | complementary DNA |
| CI | Confidence interval |
| COVID-19 | Coronavirus disease 2019 |
| CT | Cycle Threshold |
| DNA | Deoxyribonucleic acid |
| ECMO | Extracorporeal membrane oxygenation |
| HCoV 229E | Human Coronavirus 229E |
| HCoV HKU1 | Human Coronavirus HKU1 |
| HCoV NL63 | Human Coronavirus NL63 |
| HCoV OC43 | Human Coronavirus OC43 |
| ICU | Intensive care unit |
| Ig | Immunoglobulin |
| IL-6 | Interleukin-6 |
| IRP | Infectious respiratory particle |
| IQR | Interquartile range |
| JAK | Janus kinase |
| LDT | Laboratory-developed test |
| MERS-CoV | Middle East respiratory syndrome-related coronavirus |
| mRNA | Messenger RNA |
| NPS | Nasopharyngeal swab |
| NRP1 | Neuropilin -1 receptor |
| PCC | Post-COVID-19 condition |
| R0 | Basic reproduction rate |
| RNA | Ribonucleic acid |
| RR | Risk ratio |
| RT-PCR | Reverse transcription polymerase chain reaction |
| RT-qPCR | Quantitative real-time polymerase chain reaction |

| | |
|---|---|
| SAR | Secondary attack rate |
| SARS-CoV-1 | Severe acute respiratory syndrome coronavirus 1 |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| SD | Standard deviation |
| THL | Terveyden ja hyvinvoinnin laitos |
| VTM | Viral transport medium |
| WHO | World Health Organization |

# List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Laine M*, Ahti J*, Peltola V, Peri P, Hakanen AJ, Waris M. Diagnostic Efficacy and Tolerability of Molded Plastic Nasopharyngeal Swab (FinSwab) Compared to Flocked Nylon Swab in Detection of SARS-CoV-2 and Other Respiratory Viruses. *Microbiol Spectr*. 2021 Oct 31; 9(2): e0073621 *equal contribution

II Ahti J, Österback R, Keskitalo A, Mokkala K, Vidbäck S, Veikkolainen V, Vuorinen T, Peltola V, Hakanen AJ, Waris M, Laine M. Diagnostic Performance and Tolerability of Saliva and Nasopharyngeal Swab Specimens in the Detection of SARS-CoV-2 by RT-PCR. *Microbiol Spectr*. 2023 Jun 15; 11(3): e0532422

III Ahti J, Toivonen L, Ollila H, Ivaska L, Salo-Tuominen K, Vuorinen T, Lempainen J, Peltola V. Household Transmission and Clinical Features of Respiratory Tract Infections That Were SARS-CoV-2 Positive and Negative. *J Infect Dis*. 2024 Oct 15; 230(4): e837–e846

IV Ahti J, Ollila H, Toivonen L, Salo-Tuominen K, Ivaska L, Julkunen I, Peltola V. SARS-CoV-2 Seroprevalence and Seroconversion in Finnish Households with School-Aged Children between June 2020 and April 2022. Manuscript

# 1 Introduction

In November 2002, a series of severe pneumonia cases in Foshan, China, was the first sign of a new coronavirus, SARS-CoV-1, that had crossed over from animals to humans (Guan et al., 2003; Peiris et al., 2003). Then, in June 2012, in Jeddah, Saudi Arabia, a new type of coronavirus, MERS-CoV, passed from dromedary camels to humans and caused serious respiratory infections (Memish et al., 2014). Again, in December 2019, a cluster of severe pneumonia in Wuhan, China, marked the emergence of yet another coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which soon became a global pandemic (Mahase, 2020; Zhu et al., 2020). Despite the similarities in their origins, these three outbreaks had very different global consequences.

The spread of SARS-CoV-2 in early 2020 was extensive. By April 2020, global confirmed cases exceeded 3 million, and the death toll surpassed 200,000 (WHO, 2020a). As a response, the World Health Organization (WHO) implemented the "test, trace, isolate" protocol, and extreme and unprecedented actions were taken worldwide (Chung et al., 2021; WHO, 2020b). Among these actions was the significant expansion of laboratory testing (WHO, 2020c). However, the limited number of global laboratory equipment suppliers could not meet the sudden increase in demand. This resulted in a severe shortage of medical equipment, including nasopharyngeal swabs (NPS), even in high-income countries like Finland. (Decker et al., 2020; Livingston et al., 2020; Ranney et al., 2020) NPS is the golden standard of specimen collection (Hadaya et al., 2020), and as a consequence of the shortage, the "test, trace, isolate"-protocol was compromised from the outset.

Three-dimensional (3D) printing is an effective method for manufacturing small objects like NPS. Utilizing 3D printing allows for quick initiation of the manufacturing process; however, the challenge of this technique is its limited capacity for large-scale production output. (Ngo et al., 2018; Tack et al., 2016). The lack of laboratory equipment during the early days of COVID-19 pandemic prompted several studies that investigated 3D printed NPS for SARS-CoV-2 diagnostics (Alghounaim et al., 2020; Arjunan, 2021; Callahan et al., 2020; Decker et al., 2020; Oland et al., 2021). As an alternative to 3D printing, the plastic injection molding technique requires more time to initiate but is more suitable for high-volume

production of small objects like NPS (Au et al., 2014; Zema et al., 2012). However, some patients may be unsuitable for the use of NPS in sample collection, for example, due to previous rhino surgery or coagulopathies (Koskinen et al., 2021). Thus, alternative methods for sampling respiratory pathogens aside from NPS have been investigated (Lee Rose et al., 2021; Nagura-Ikeda et al., 2020; Poukka et al., 2021; Teo et al., 2021; Wyllie et al., 2020; Wölfl-Duchek et al., 2022). Saliva collection is often convenient and well-tolerated, can be easily self-administered, and has demonstrated comparable efficacy to NPS (Bastos et al., 2021; Butler-Laporte et al., 2021; Callahan et al., 2021; Uršič et al., 2022; Williams, Bond, Zhang, et al., 2020).

In the "test, trace, isolate" protocol, contacts of SARS-CoV-2-positive persons were traced and then isolated to their households to prevent the spread of the virus (WHO, 2020b). However, the methods of isolating individuals within their households are limited, making households a significant location for SARS-CoV-2 transmission (Madewell et al., 2021). The transmission rate of SARS-CoV-2 in households can vary significantly based on factors like the SARS-CoV-2 variant, household mask usage, vaccination coverage, and the demographics of the household population. (F. Chen et al., 2022; Chu et al., 2021; Fiolet et al., 2022; Gomaa et al., 2021; Jørgensen et al., 2022; Lewis et al., 2021; Madewell et al., 2021, 2022; Paul et al., 2021; Thompson et al., 2021)

Children play a key role in the spread of respiratory viruses in households (Schlinkmann et al., 2018; Worby et al., 2015), and understanding SARS-CoV-2 transmission in households with children is essential for controlling the spread. Despite limited information on children's role in transmission of SARS-CoV-2 in the early days of the pandemic, strict restrictions were imposed on the child population. However, these restrictions can negatively impact the development of children and may have long-lasting consequences (Gadermann et al., 2021; Kauhanen et al., 2023; Kuehn et al., 2024; Singh et al., 2020). While the role of children in transmitting SARS-CoV-2 has gained more significance with the emergence of new variants, children do not appear to be the primary drivers of the pandemic (F. Chen et al., 2022; Goldstein et al., 2021; Jørgensen et al., 2022; Madewell et al., 2022).

This doctoral thesis examines the transmission of SARS-CoV-2 and other respiratory pathogens in Finnish households with children. Additionally, the thesis investigates the efficacy of nasopharyngeal swabs produced using plastic injection molding technique and the utilization of saliva in the detection of SARS-CoV-2.

# 2 Review of the Literature

## 2.1 Human coronaviruses

Coronaviruses are a group of large, single-stranded, enveloped, positive-sense RNA viruses (Masters, 2006). Their surfaces are covered with characteristic surface projections that extend outward like tall mushrooms. This unique appearance resembles a solar corona when viewed through an electron microscope, from which their name derives (Tyrell et al., 1968).

Coronaviruses are classified under the order *Nidovirales*, family *Coronaviridae*, subfamily *Orthocoronavirinae*, and include the genera *alpha-*, *beta-, gamma-* and *deltacoronaviruses (ICTV, 2024).* Aside from infecting humans, *alpha-* and *betacoronaviruses* can infect various wild and domestic animals, including bats, pigs, cattle, chicken, mice, cats, dogs, deer, and hedgehogs (Chan et al., 2013; Corman et al., 2018), with bats playing a most significant role as reservoir hosts (Tang et al., 2022). Coronaviruses can be transmitted to humans from these animal hosts, resulting in zoonotic diseases that can lead to significant illness in humans due to the absence of prior immunity. In humans, coronaviruses cause respiratory tract infections that range from mild illnesses to severe, potentially fatal diseases (Bradburne et al., 1967; Memish et al., 2014; Peiris et al., 2003; Zhu et al., 2020).

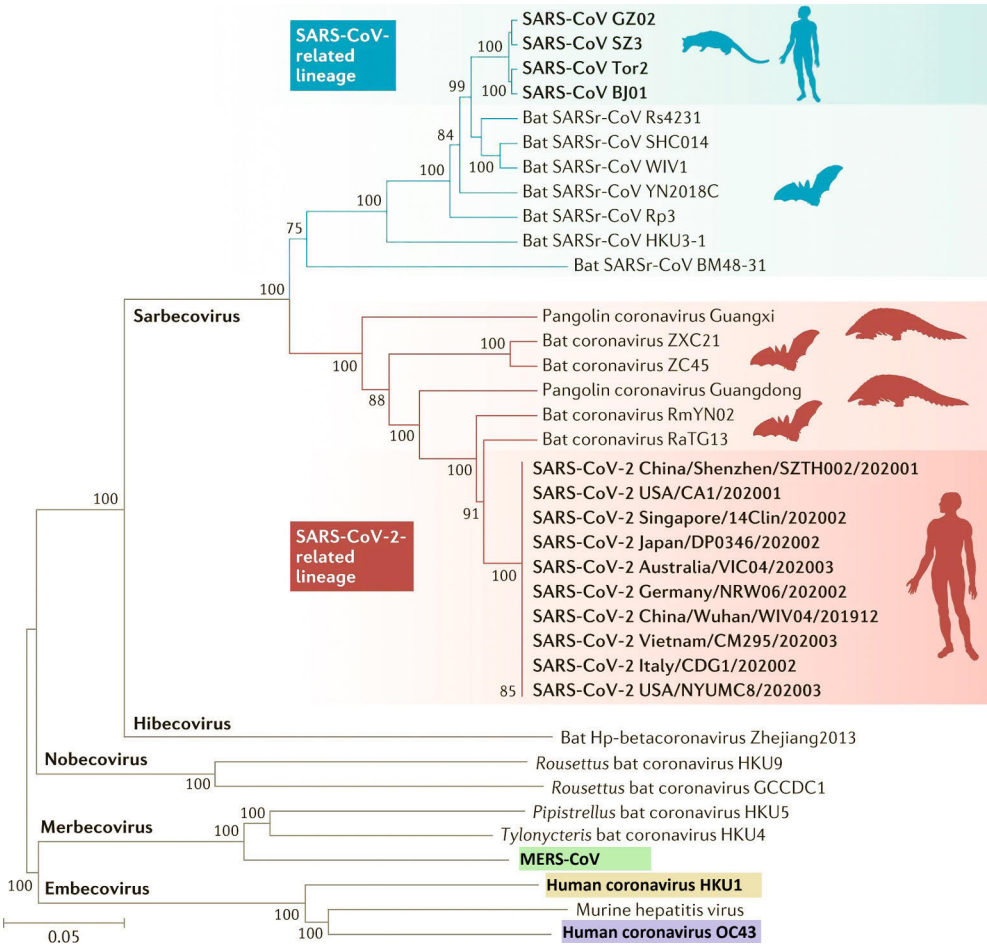


**Figure 1.** Phylogenetic tree of SARS-CoV-2 and other related *betacoronaviruses*. Modified and reproduced from Hu et al. Nat Rev Microbiol 2021, with the permission of the copyright holders.

### 2.1.1 Seasonal coronaviruses

Seven human coronaviruses have been identified. Four of them include human coronaviruses 229E (HCoV 229E), OC43 (HCoV OC43), NL63 (HCoV NL63), and HKU1 (HCoV HKU1), which typically cause mild respiratory tract disease and can be categorized as seasonal coronaviruses based on their seasonal emergence primarily during winter months (Bradburne et al., 1967; Gaunt et al., 2010; Monto et al., 2020). These seasonal coronaviruses are a significant contributor to the common cold, accounting for 10-15% of cases (Nickbakhsh et al., 2020).

Some theories propose that the transition of coronaviruses from zoonotic origins to humans may have initially led to significant epidemics or even pandemics,

resulting in severe disease manifestations (Brüssow & Brüssow, 2021; King, 2020). Over time, these viruses appear to have diminished in pathogenicity, ultimately becoming seasonal. Most attention has been directed towards the Russian Flu pandemic of 1889-1890, which is estimated to have caused approximately 1-2 million fatalities (Berche, 2022; Erkoreka et al., 2022). Molecular clock analyses suggest that the zoonotic transmission of human coronavirus OC43 likely occurred around 1890 (Vijgen et al., 2005), and the clinical characteristics observed during the Russian Flu resemble those of coronavirus disease 2019 (COVID-19) (Brüssow & Brüssow, 2021; Erkoreka et al., 2022; King, 2020). Nevertheless, many aspects of Russian flu also align with influenza viruses, and the precise causative agent of 1889-1890 pandemic is still unidentified (Saunders-Hastings & Krewski, 2016).

### 2.1.2 Emerging coronaviruses

The other three identified human coronaviruses, in the order of their appearance in humans, are severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), Middle East respiratory syndrome-related coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These "emerging coronaviruses" have all appeared in humans in the 21st century and potentially cause severe disease manifestations. (Memish et al., 2014; Peiris et al., 2003; Zhu et al., 2020)

#### 2.1.2.1 Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1)

SARS-CoV-1, previously known as SARS-CoV, is a *betacoronavirus* and was the first coronavirus identified as causing severe disease and significant public health events. It was first noted in November 2002 as a series of severe pneumonia cases in Foshan, China (Peiris et al., 2003). Evidence suggests that the virus was introduced to humans through civets, with bats serving as a natural reservoir for the virus (Guan et al., 2003; Hu et al., 2017).

The spread of SARS-CoV-1 was rapid but relatively contained, with fewer than 9000 cases reported. Affected areas, in addition to China, included Asia-Pacific countries and Canada. However, the disease caused by SARS-CoV-1 is severe, resulting in a fatality rate of approximately 11% (Chan-Yeung & Xu, 2003). No outbreaks of SARS-CoV-1 have been reported since 2004 (WHO, 2016).

#### 2.1.2.2 Middle East respiratory syndrome–related coronavirus (MERS-CoV)

MERS-CoV, initially called novel coronavirus 2012, is a *betacoronavirus* that can lead to severe disease. It was first encountered in Jeddah, Saudi Arabia, in 2012 (Memish et al., 2014). Since then, there have been 2613 laboratory-confirmed cases and 943 deaths as of May 2024, resulting in a fatality rate of 36% (WHO, 2024a). While MERS primarily occurs in the Middle East, several cases have also been reported in Europe, the USA, and Asia (Memish et al., 2020). In contrast to SARS-CoV-1, MERS-CoV cases continue to emerge sporadically (WHO, 2024a).

The zoonotic transmission of MERS to humans is primarily attributed to dromedary camels, and camel-to-human interaction remains the principal route of infection, although human-to-human transmission does occur (Memish et al., 2020). While bats are suspected to be the natural reservoir for the virus, MERS-CoV was detected in dromedary camels in 1983, suggesting that the virus has been in circulation among these animals for at least 30 years prior to its transmission to humans (Müller et al., 2014).

#### 2.1.2.3 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

SARS-CoV-2, the virus responsible for COVID-19, is a *betacoronavirus* within the subgenus *Sarbecovirus,* species *Betacoronavirus pandemicum.* Its closest relative among human coronaviruses is SARS-CoV-1 (ICTV, 2024). At the scale of the entire genome, SARS-CoV-2 demonstrates a 79% sequence identity to SARS-CoV-1 and a 50% identity to MERS-CoV (R. Lu et al., 2020). However, numerous *betacoronaviruses* found in bats, particularly in intermediate horseshoe bats (*Rhinolophus affinis*), exhibit a significantly higher degree of similarity to SARS-CoV-2. RaTG13 coronavirus stands out with the highest similarity, displaying 96.1% nucleotide identity to SARS-CoV-2. (P. Zhou et al., 2020).

SARS-CoV-2, as well as other coronaviruses, comprises four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (**Figure 2a)**. The nucleocapsid protein (N) is responsible for encapsulating the RNA genome, while the spike (S), envelope (E), and membrane (M) proteins collectively form the viral envelope (Brian & Baric, 2005; M.-Y. Wang et al., 2020).

The most significant distinctions between SARS-CoV-2 and its close relatives are observed in the spike protein gene (P. Zhou et al., 2020). The spike (S) protein of SARS-CoV-2 is composed of two functional parts: S1 and S2. Upon entry into host cells, these subunits are cleaved at specific cleavage sites, facilitating entry. (M.-Y. Wang et al., 2020) SARS-CoV-2 spike proteins are cleaved at two sites: S1/S2 and S2′ (Walls et al., 2020). Notably, SARS-CoV-2 differs from SARS-CoV-1 and

other related viruses in that it possesses a multibasic cleavage site at S1/S2. This feature allows cleavage by a broader range of proteases, including furin, TMPRSS2, and other proteases. (Essalmani et al., 2022; Hoffmann, Kleine-Weber, & Pöhlmann, 2020; Walls et al., 2020) In contrast, viruses with monobasic cleavage sites are cleaved by specific proteases such as trypsin (Belouzard et al., 2009). The presence of multibasic cleavage sites in other viral entities is associated with increased transmissibility (Steinhauer, 1999).

SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptor to gain entry into host cells (Walls et al., 2020). ACE2 is a protein situated in the human cell membrane that plays a crucial role in the regulation of blood pressure via the renin-angiotensin-aldosterone system (Donoghue et al., 2000).

Upon entering the target cell, the S1 subunit of SARS-CoV-2 binds to the ACE2 receptor present on the target cell surface. Subsequently, the enzyme TMPRSS2 cleaves the protein, thereby exposing the S2 domain, which is crucial for the fusion with the host cell membrane (Hoffmann, Kleine-Weber, Schroeder, et al., 2020) (**Figure 2b)**. Additionally, the S1 fragment interacts with the neuropilin-1 receptor (NRP1), further facilitating cell entry (Cantuti-Castelvetri et al., 2020; Daly et al., 2020). Following cell entry, the production of viral proteins starts with the synthesis of replicase proteins. Transcription occurs in double-membrane vesicles. In addition to producing double-stranded RNA, a discontinuous viral transcription process generates subgenomic negative-sense RNAs. (Kim et al., 2020; V'Kovski et al., 2021) Subsequently, viral particles are assembled within the endoplasmic reticulum and the Golgi apparatus, before being released via exocytosis in secretory vesicles (V'Kovski et al., 2021).

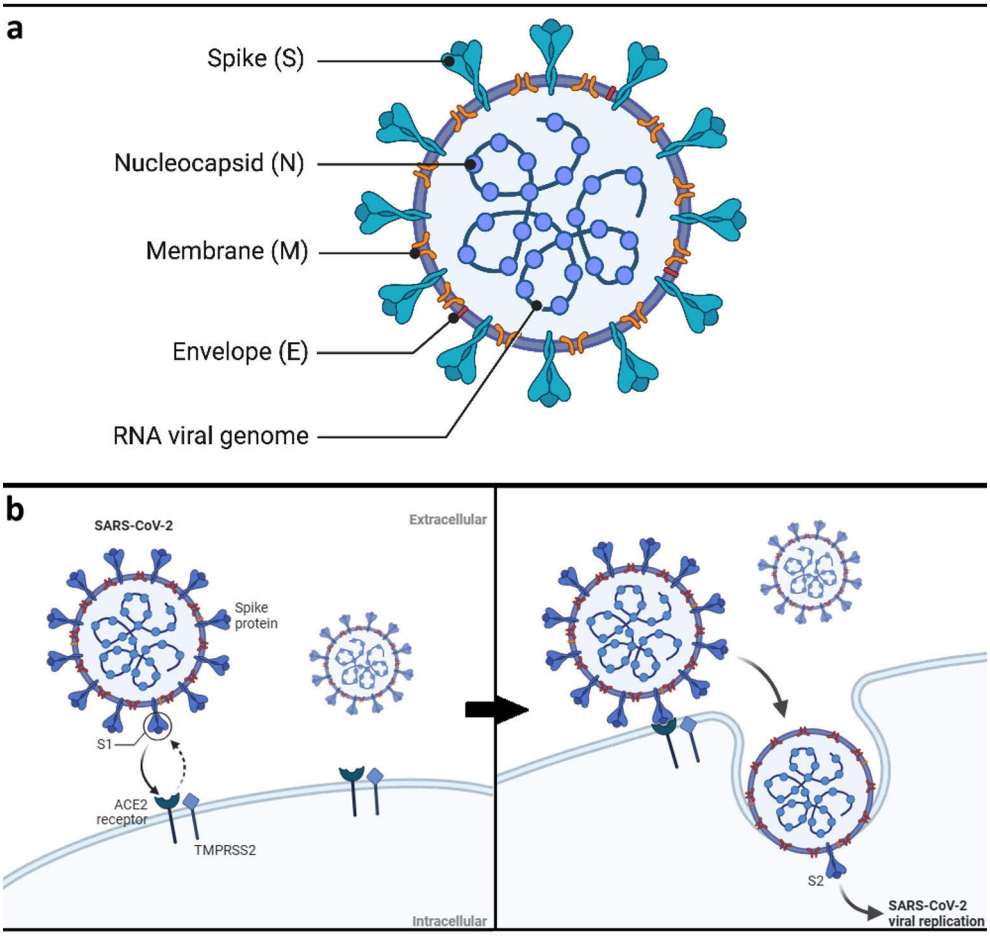


**Figure 2**. **a**) Structure of SARS-CoV-2 and **b**) Invasion mechanism of SARS-CoV-2. Created with BioRender.com

The exact mechanisms through which SARS-CoV-2 transitioned to become a human coronavirus have yet to be elucidated (Alwine et al., 2023). Bats are widely regarded as the natural reservoir, given the extensive diversity of coronaviruses found within various bat species, including RaTG13, the closest known relative to SARS-CoV-2 (Zhou et al., 2021). Nonetheless, the observed genetic dissimilarities of 3.9% between the two genomes suggest that RaTG13 is probably not the most immediate ancestor of SARS-CoV-2 (Alwine et al., 2023). The involvement of intermediate hosts such as Malayan pangolins (*Manis javanica*) has been proposed since coronaviruses with similar receptor binding domains have been identified in these animals (Lam et al., 2020). Additionally, sequences of SARS-CoV-2 were found in the cages and stalls housing live animals at the Wuhan Market. However,

no live animals were reportedly sampled, and the immediate precursor remains to be discovered. (Worobey et al., 2022)

Alternative theories of origin, including the possibility of a laboratory incident, have been investigated. However, early isolates of the virus do not display signs of human engineering, and mutations that facilitate adaptation in common animal models such as mice appear to be absent. Additionally, the Wuhan Institute of Virology, the institute that has been suspected in media regarding laboratory leaks, has reportedly cultured SARS-CoV-related viruses that show closer genetic relationships to SARS-CoV-1 than to SARS-CoV-2. Nonetheless, the lack of transparency from the Chinese government has fuelled these speculations, despite the evidence pointing in the opposite direction. (Alwine et al., 2023; Holmes et al., 2021; Worobey et al., 2022) Current evidence strongly supports the hypothesis of zoonotic transmission to humans, originating from bats and potentially involving intermediate hosts (Lam et al., 2020; Tang et al., 2022; P. Zhou et al., 2020).

## 2.2 Epidemiology

The first identified outbreak of COVID-19, the disease caused by SARS-CoV-2, began in Wuhan, China, in December 2019 (Zhu et al., 2020). More precisely, geographical clustering and analyses of environmental samples indicate that the Huanan Seafood Market was a specific focal point for initial transmission (Worobey et al., 2022), and molecular clock analyses pinpoint the first human-to-human transmission occurred in October-November 2019 (Pekar et al., 2021). Extensive spread of the virus in human-to-human transmission was confirmed in family clusters in January 2020 (Chan et al., 2020). International spread was imminent, and cases were soon reported from Japan, Korea, and Thailand (C. Wang et al., 2020). Wuhan was placed under lockdown on January 23rd, a Public health emergency of international concern was announced by WHO on January 30th, and the COVID-19 pandemic was declared on March 11$^{th}$ (Durrheim et al., 2020; Mahase, 2020).

### 2.2.1 Coronavirus disease 2019 (Covid-19) pandemic

The term "pandemic" is derived from the ancient Greek components "pan," meaning "all," and "demos," signifying "people." It refers to an epidemic that substantially impacts a significant number of individuals across the globe (Morens et al., 2009). By the time COVID-19 was declared a pandemic, it had reportedly spread to 114 countries, including 118,000 cases and 4291 fatalities (WHO, 2023a). By April 29$^{th}$, 2020, globally confirmed cases exceeded 3 million and deaths 200,000 (WHO,

2020b). The timeline of weekly cases and fatalities in Finland, the USA, and Europe in 2020-2022 are shown in **Figure 3**. During the first months of the pandemic, the distribution of global COVID-19 cases varied significantly among nations. For instance, Italy, which experienced a severe outbreak, reported 195,351 cumulative cases and 26,384 fatalities as of April 26, 2020. In contrast, Finland, with a relatively contained situation, documented 5,521 cumulative cases and 282 fatalities during the same timeframe (WHO, 2023a). Furthermore, the reporting of cases and fatalities was insufficient during the initial months of the pandemic due to the lack of medical equipment, including testing tools (Li et al., 2020; Ranney et al., 2020; Sharfstein et al., 2020).

As a result of the extensive spread of SARS-CoV-2, various interventions were implemented to contain the spread, including the partial suspension of both international and domestic travel and the closure of schools and other public facilities (Liu et al., 2021; WHO, 2021). By 29$^{th}$ November 2024, the cumulative incidence of reported COVID-19 cases has exceeded 750 million, with the death toll surpassing 7 million (WHO, 2023a). However, the actual number of cases and fatalities is likely higher since a major number of infections were undetected, especially in the early months of the pandemic (Li et al., 2020; Russell et al., 2020).

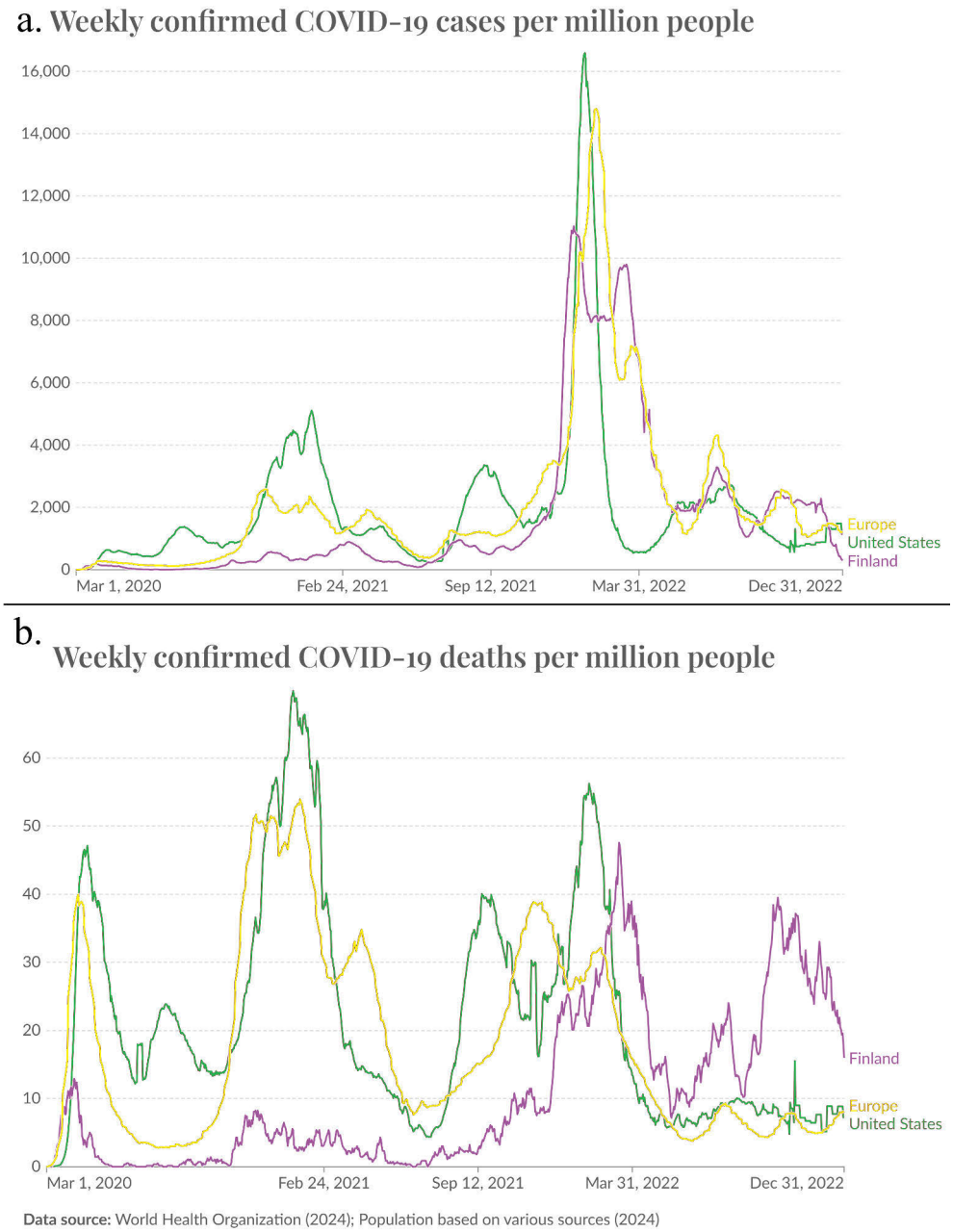


**Figure 3.** Weekly confirmed COVID-19 cases (**a**) and COVID-19 deaths (**b**) per million people in Finland, Europe, and the United States. Timeline based on WHO data. Reproduced with permission from copyright owners (CC BY).

#### 2.2.1.1 Pandemic in Finland

The transmission of SARS-CoV-2 remained relatively controlled in Finland until late 2021 (WHO, 2023a) (**Figure 3**). According to WHO data, 39,086 cases and 618 deaths were reported by December 31st, 2020. Cases exceeded 100,000 (986 deaths) on July 6th and 200,000 (1497 deaths) on November 27th, 2021. By April 5th, 2022, cases exceeded 1 million, and fatalities 4200.

Between 2020 and 2022, Finland implemented various non-pharmaceutical measures to contain COVID-19. From March to May 2020, November 2020 to June 2021, and September 2021 to January 2022, gatherings were restricted, with limits ranging from 10 to 500 people. Outdoor gatherings had looser restrictions. Recommendation for remote work was in effect from March 2020 until Spring 2022. Face masks were recommended starting in November 2020. Lower (grades 7-9) and upper secondary schools (high schools and vocational schools), and universities switched to remote learning from March 8 to April 11, 2020, while first grades (grades 1-3) of primary schools attempted to continue normally. Throughout autumn 2020 to spring 2021, schools occasionally shifted to remote learning in response to increased case numbers. Regular school operations resumed in autumn 2021, apart from a facemask recommendation that was continued until spring 2022 (Johanson et al., 2025).

### 2.2.2 Variants of SARS-CoV-2

The emergence of various variants of concern greatly influenced the trajectory of the COVID-19 pandemic. The first evidence of a wild-type virus adapting to humans was observed through the population-level substitution of the 614D allele with the 614G allele between March to May 2020 (Koelle et al., 2022). Higher viral loads compared to 614D and widespread transmission of SARS-CoV-2 during the rise of the 614G variant also indicated increased transmissibility. However, the COVID-19 severity remained consistent. (Volz et al., 2021) The summary of variants of concern and their features is presented in **Table 1**.

**Table 1.** SARS-CoV-2 variants of concern, location and time of first detection, transmissibility, severity, and vaccine effectiveness. Comparisons are to wild-type variant. Composed based on the articles of Manathunga et al. Virol J 2023, Grint et al. Eurosurveillance 2021, Koelle et al. Science 2022, Butt et al. JAMA Intern Med 2022, Zeng et al. BMC Medicine 2022, Polack et al. NEJM 2020. R0 refers to the relative reproduction number compared to wild-type variant. R0 represents the average number of secondary infections produced by a single infected individual in a fully susceptible population.

| Variant | Detected (Time, Country) | Transmissibility (R0) | Severity | Vaccine Effectiveness (%) |
|---|---|---|---|---|
| Wild-Type | December 2019, China | Reference (1) | Reference | Reference (100) |
| Alpha | September 2020, UK | Slightly Increased (1.22) | Slightly Increased | Slightly reduced (93) |
| Beta | September 2020, South Africa | Slightly Increased (1.19) | Slightly Increased | Reduced (77) |
| Gamma | November 2020, Brazil | Slightly Increased (1.21) | Slightly Increased | Reduced (66) |
| Delta | December 2020, India | Increased (1.38) | Increased | Reduced (82) |
| Omicron | November 2021, South Africa | Greatly Increased (1.90) | Decreased | Greatly reduced (59) |

The Alpha variant (B.1.1.7), first identified in the United Kingdom in late 2020, was the initial variant to demonstrate significant increases in transmissibility (Davies et al., 2021) and disease severity (Grint et al., 2021). The emergence of Alpha was soon followed by the detection of the Beta (B.1.351) variant in South Africa and the Gamma (P.1) variant in Brazil (Koelle et al., 2022). Both variants showed higher infection rates, even among populations with a significant prevalence of prior infections with earlier variants, further indicating the immune evasion potential of SARS-CoV-2 variants (Mwenda et al., 2021; Sabino et al., 2021).

The Delta variant (B.1.617.2) was first discovered in India in December 2020. It swept rapidly through India in Spring 2021 and soon became the dominant variant globally (Dhar et al., 2021). The Delta variant was characterized by enhanced transmissibility and a diminished level of immune protection by prior SARS-CoV-2 infections (Allen et al., 2022). Additionally, the reduced effectiveness of COVID-19 vaccinations and increased disease severity were observed (Butt et al., 2022; Fiolet et al., 2022).

The emergence of the Omicron variant (B.1.1.529) in November 2021 in South Africa marked a new phase in the pandemic (Viana et al., 2022). The Omicron variant swiftly demonstrated its superior transmissibility by becoming a globally dominant variant within just a few weeks of its discovery (Koelle et al., 2022). The Omicron variant exhibited over 30 mutations that enhanced its ability to evade existing immunity and facilitate transmission between individuals, superiorly compared to prior variants (Baker et al., 2022; Elliott et al., 2022; Liu et al., 2022; Madewell et al., 2022; Manathunga et al., 2023; Viana et al., 2022). However, the severity of disease associated with the Omicron variant was significantly lower compared to the Delta

variant (Lewnard et al., 2022). This reduction in severity compared with progression of vaccinations prompted a relaxation of contact restrictions, which also contributed to an increase in community transmission. Additionally, decreased vaccine effectiveness against symptomatic infections was reported, while effectiveness against severe disease and death remained high, with fatalities from Omicron predominantly occurring among unvaccinated individuals (Andrews, Stowe, et al., 2022; Koelle et al., 2022; Lewnard et al., 2022; Zeng et al., 2022). Even though the WHO declared a COVID-19-related global health emergency to be over in May 2023 (Lenharo, 2023), SARS-CoV-2 continues to evolve (Yajima et al., 2024).

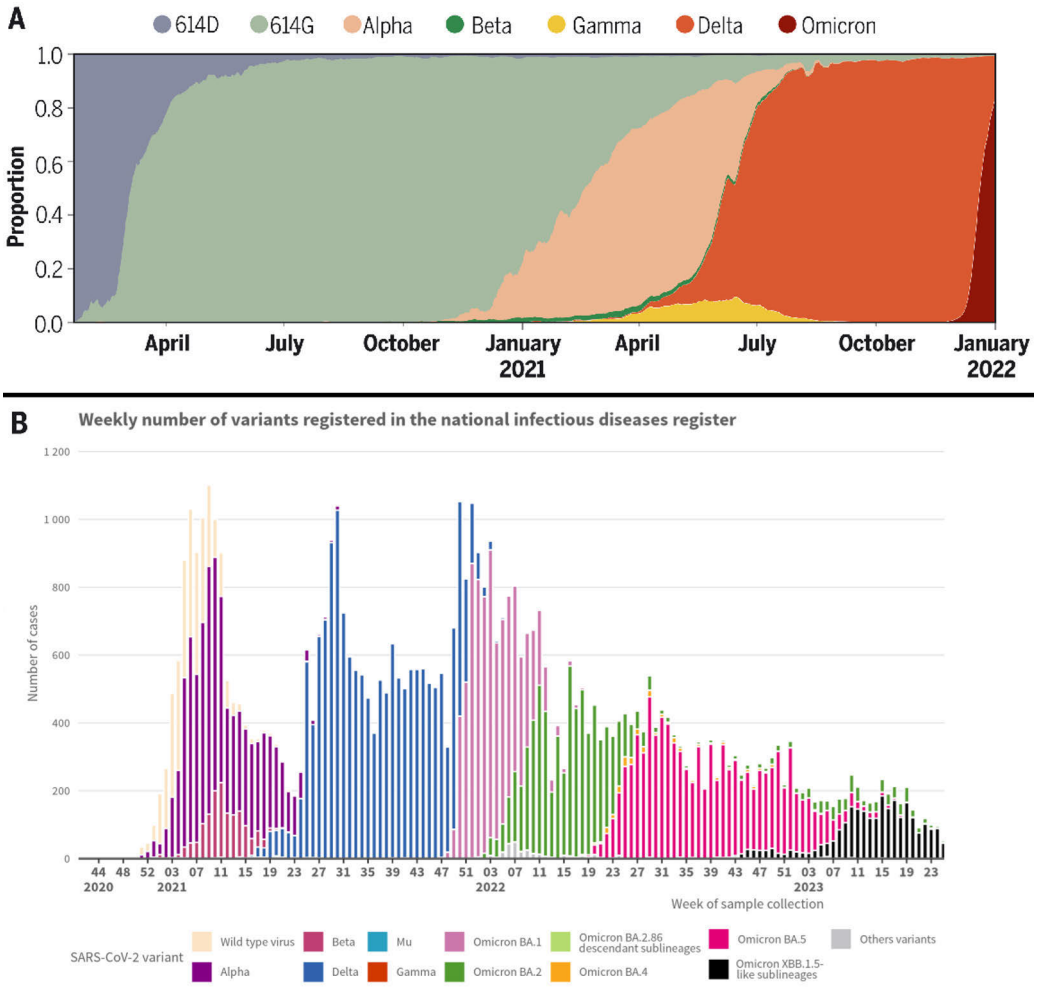


**Figure 4**. Emergence of SARS-CoV-2 variants during the pandemic. **A**) Global proportions, and **B**) Number of detected variants in Finland. Modified and reproduced from Koelle et al., Science 2022, and THL.fi, with the permission of the copyright holders.

## 2.3 Transmission

### 2.3.1 Transmission mechanisms

The initial transmission mechanism from a zoonotic source to humans remains unidentified. However, human-to-human transmission was confirmed within the initial weeks of the COVID-19 outbreak, particularly among family clusters (Chan et al., 2020).

The principal route for transmission between humans is via direct deposition of droplets produced by coughing, sneezing, and speaking (Anfinrud et al., 2020; Harrison et al., 2020; WHO, 2024b). Transmission also occurs through air-borne aerosols that are produced during breathing, speaking, or, more effectively, singing. (Echternach et al., 2020; Jarvis, 2020). Since 2024, the WHO has recommended using the term *infectious respiratory particle* (IRP) to cover all infectious airborne particles, regardless of the size of the particle (WHO, 2024b). Respiratory droplets can transmit the virus up to two meters, with the distance influenced by the intensity of a cough or the volume of speech (Harrison et al., 2020; Jarvis, 2020). Aerosols are transported by air currents, allowing them to travel much greater distances (Wei & Li, 2016). However, the concentration of aerosols is reduced by dispersion, showing a 7-fold reduction from one meter to two meters away from the source (Jarvis, 2020). The study suggests that SARS-CoV-2 can remain infective in aerosols for hours under the right circumstances (Van Doremalen et al., 2020).

SARS-CoV-2 may be transmitted through direct contact, such as handshakes, as well as through indirect transmission via contaminated surfaces, including door handles (Riddell et al., 2020; Zhang et al., 2024). Nonetheless, the efficacy of these transmission routes appears to be minimal in comparison to the transmission that occurs through respiratory droplets and aerosols (National Center for Immunization and Respiratory Diseases, 2020). Although SARS-CoV-2 RNA has been detected in fecal samples of infected individuals, there is no evidence of fecal-oral transmission (Termansen & Frische, 2023; Xu et al., 2020). **Figure 5.** summarizes the routes of transmission.

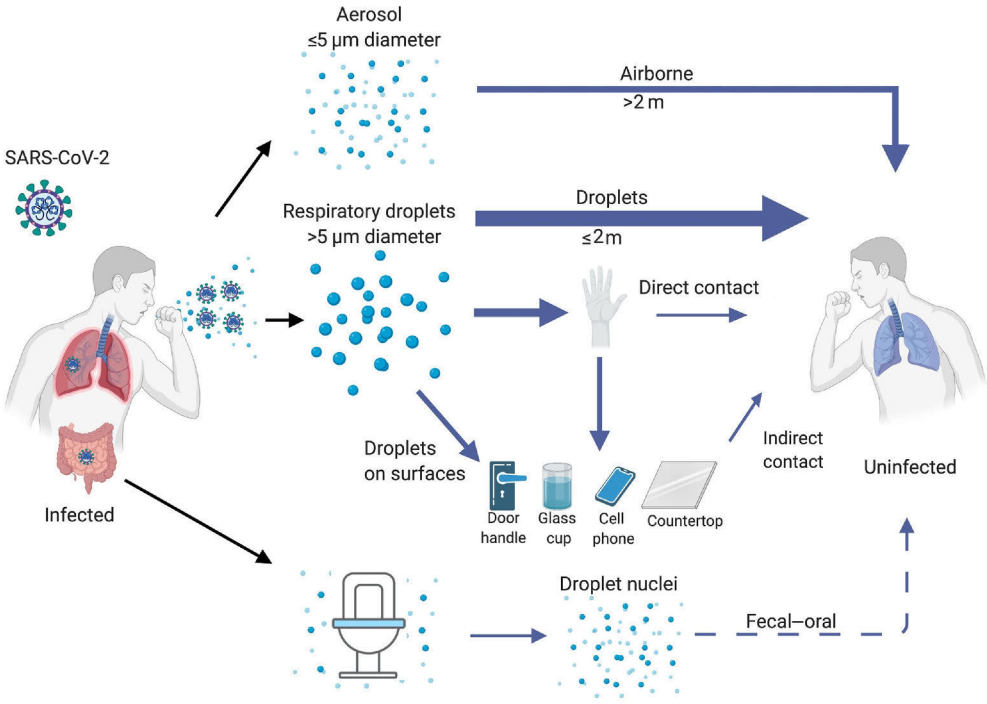


**Figure 5.** SARS-CoV-2 transmission routes. Solid arrows represent the transmission pathways originating from an infected individual, with a diminishing width of the arrows indicating the relative significance of each pathway. The dashed lines along the fecal-oral route illustrate the potential for transmission, although the fecal-oral transmission has not been confirmed in epidemiological studies. Modified and reproduced from Harrison et al. Trends in Immunology 2020, with the permission of the copyright holders.

### 2.3.2 Community transmission

Transmission generally occurs in places where people gather. The likelihood of transmission increases when a larger number of individuals assemble in confined spaces, especially if there is inadequate ventilation (Hunziker, 2021; Prather et al., 2020). Activities that promote the dispersal of respiratory droplets and aerosols, such as singing, further elevate the risk of transmission (Katelaris et al., 2021). However, the risk of transmission in outdoor settings remains low (Bulfone et al., 2021). The comparison of transmissibility among various viruses presents significant challenges, as transmission is influenced by numerous factors within communities.

Most commonly used metric to assess transmission is the basic reproduction rate (R0) that represents the average number of secondary infections produced by a single infected individual in a fully susceptible population. Available studies estimate that the R0 of SARS-CoV-2 ranges from approximately 1.5 to 3. The R0 value is influenced by the specific variant in circulation and the demographics of the studied

population, with reported values extending from 1.20 to as high as 14.8 reported from the Diamond Princess cruise ship in Japan (Billah et al., 2020; Manathunga et al., 2023). These R0 values are significantly higher than those of SARS-CoV-1 (0.58), MERS-CoV (0.68), and seasonal influenza (1.28) (Abdelrahman et al., 2020). Moreover, the R0-values for pandemic influenza from the years 1918 (1.80), 1957 (1.65), 1968 (1.80), and 2009 (1.46) present comparable figures (Biggerstaff et al., 2014). However, the comparisons of R0 values may be uneven due to the substantial increase in both international and domestic travel since the 20th century, which has a considerable impact on reproduction rates. Nevertheless, there are viruses with significantly higher transmissibility than SARS-CoV-2. For example, the basic R0 value for measles is estimated to be between 12 and 18, with a reported range extending from 1.4 to 770 (Guerra et al., 2017).

Although social restrictions may effectively decrease human interactions and subsequently lower the transmission of viruses in public environments, such measures are not as feasible within private households, leading to households being a significant source of SARS-CoV-2 transmission (Thompson et al., 2021). Still, reducing face-to-face contact within households during COVID-19 infection lowers transmission rates (Chu et al., 2021). A study on SARS-CoV-2 transmission by Baker et al. demonstrated that when the infected index person isolated or at any point wore a mask at home during the infection, the secondary transmission to other household members was significantly reduced (68% to 41%, and 69% to 40%, respectively) (Baker et al., 2022). Increased transmissibility within households is also associated with the duration of exposure, the symptomatic status of the index case, and the comorbidities of the secondary case (Lewis et al., 2021; Madewell et al., 2021; Thompson et al., 2021).

Household transmission rates are frequently represented by secondary attack rates (SARs) of the infection. The SAR is defined as the ratio of secondary infections occurring among non-index household members to the total number of non-index household members. Household transmission of the emerged SARS-CoV-2 variants corresponds to the transmissibility and vaccine effectiveness reported in chapter 2.2.2, with the Omicron variant showing higher SAR compared to other variants (F. Chen et al., 2022; Madewell et al., 2022). In a comprehensive systematic review and meta-analysis, Madewell et al. additionally investigated the efficacy of vaccines in mitigating both infectiousness in individuals who are infected and susceptibility to infection. Vaccine effectiveness on infectiousness was 75% for Alpha, 22% for Delta, and 18% for Omicron. Vaccine effectiveness on susceptibility was 79% for Alpha, 56% for Delta, and 18% for Omicron (Madewell et al., 2022).

The influence of children on the transmission of SARS-CoV-2 has become increasingly significant following the emergence of new variants of the virus. In relation to the wild-type variant, studies have indicated that children exhibit both

reduced susceptibility and lower transmissibility of the virus when compared to adults, while younger children show greater infectivity than older children (F. Chen et al., 2022; Madewell et al., 2020; Paul et al., 2021; Thompson et al., 2021). However, with more recently emerged variants, children and adults demonstrate equal susceptibility and infectivity (Baker et al., 2022; F. Chen et al., 2022; Chun et al., 2022).

The SARs of different viruses across various studies are hardly comparable as SAR varies considerably among study populations, even when the same virus is being examined (F. Chen et al., 2022; Gomaa et al., 2021; Jørgensen et al., 2022; Madewell et al., 2020). Nukiwa-Souma et al. examined the role of children in seasonal influenza and discovered that the SAR of influenza-like illness was highest among children 1-4 years old (20%), while SAR in 5-9 years old (11%) and overall SAR (6%) were lower. However, the index case characteristics demonstrated no significant impact on SAR (Nukiwa-Souma et al., 2012). A study by Sugimoto et al. examined the transmission of the 2009 A/H1N1 pandemic influenza in a youth camp. The outbreak resulted in a secondary attack rate (SAR) of 42% among campers in shared cabins. In contrast, the household SAR following the camp was 11% for children at home and 4% for adults at home. The results suggest that the timing of the exposure was a major contributor to secondary attacks, and children were more susceptible to the virus (Sugimoto et al., 2011). Additionally, for rhinovirus, transmission rates have been demonstrated to be higher in child index cases among siblings (100% positive) than in parents (50% positive) (Peltola et al., 2008).

## 2.4 Clinical manifestations

COVID-19 is associated with a diverse array of symptoms in individuals. Primarily, it manifests with upper respiratory tract symptoms, including rhinorrhea, sore throat, and cough (Chen et al., 2020; Esper et al., 2023; Menni et al., 2022). Additionally, fever and the loss of taste and smell are commonly recognized symptoms characteristic of COVID-19 (Chen et al., 2020; Giacomelli et al., 2020; Menni et al., 2022). Nevertheless, the virus can also impact nearly every system within the human body, affecting the lungs, gastrointestinal tract, and nervous system, among others (COVID-ICU Group, 2021; Mao et al., 2020; Pan et al., 2020). The severity of symptoms may range from asymptomatic cases to severe, potentially fatal conditions. Moreover, variants of the virus present differing proportions of symptoms and, critically, differ in morbidity and mortality (**Table 1.**). (Butt et al., 2022; Chun et al., 2022; Esper et al., 2023; Menni et al., 2022; Relan et al., 2023; Suzuki et al., 2022; Yu et al., 2022)

The incubation period, which is the time from exposure to symptom onset, ranges from 1 to 19 days, averaging 4 to 9 days. This period has been observed to

decrease gradually with the emergence of new variants. The most recently emerged Omicron variant has a mean incubation period of 3.4 days, and the first variant to emerge, the Alpha variant exhibited a mean incubation period of 5.0 days (Wu et al., 2022). The period of infectiousness lasts for a median of 5 days, although there is considerable variability. Nonetheless, most individuals do not excrete the virus one week after the onset of symptoms. (Hakki et al., 2022)

### 2.4.1 Risk factors and complications

The underlying medical conditions such as diabetes, immunosuppression or renal insufficiency, and old age are the most important prognostic factors for severe COVID-19 (Zhang et al., 2020). Additionally, male sex, obesity, and lack of infection- or vaccine-induced immunity are widely recognized as risk factors for severe COVID-19 (COVID-ICU Group, 2021; Lewnard et al., 2022; Zhang et al., 2020).

WHO defines the disease severity of COVID-19 as follows: Severe COVID-19 is defined by oxygen saturation <90% on room air, signs of pneumonia, or signs of severe respiratory distress; Non-severe COVID-19 is defined as the absence of severe COVID-19 (WHO, 2022). Severe manifestations of COVID-19 are distinguished by the involvement of organ systems aside from the upper respiratory tract. The respiratory system is the most commonly affected, resulting in viral pneumonia and, in more critical cases, acute respiratory distress syndrome (ARDS) (Chen et al., 2020; COVID-ICU Group, 2021; Zhang et al., 2020). Severe manifestations typically appear approximately one week after the initial onset of symptoms, with dyspnea, hypoxemia, raised inflammatory markers, and abnormalities in thorax x-ray being the most prominent clinical indicators (Berlin et al., 2020; F. Zhou et al., 2020). The involvement of the lower respiratory tract and/or other organ systems often leads to hospital admissions and necessitates the use of oxygen therapy. This occurrence was more prevalent prior to the emergence of the Omicron variant (Esper et al., 2023). The failure of two or more organ systems can ultimately lead to a condition referred to as multiorgan failure. The mortality of these patients is high despite treatment or intensive care unit (ICU) admission (COVID-ICU Group, 2021).

The proportion estimates of severe COVID-19 (2-23%) and case fatality (0.5-6%) depend on the definition of severe COVID-19, SARS-CoV-2 variant, and demographic characteristics of the study population (Esper et al., 2023; Lewnard et al., 2022; Li et al., 2021). A study by Esper et al. in Ohio, USA, involving primarily middle-aged individuals (age interquartile range 27.5 to 55.8 years), reported an overall hospitalization rate of approximately 10%. The rates for oxygen therapy, ICU admissions, and case fatalities were 6.3%, 2.5%, and 1%, respectively. The Omicron

variant caused lower hospitalization (5.9%), oxygen therapy (3.4%), and ICU admission rates (1%). (Esper et al., 2023) However, the estimates regarding the prevalence of severe cases may be inflated, as asymptomatic and mild instances often go undiagnosed (Joung et al., 2022; Suzuki et al., 2022; Wang et al., 2023). Furthermore, studies may exclude cases of low positive SARS-CoV-2 results from their analyses. For instance, Esper et al. excluded positive specimens with cycle threshold values >30 (Esper et al., 2023).

COVID-19 can also be complicated by various secondary bacterial or fungal infections, including bacterial pneumonia and otitis media; however, the incidence of these complications is generally considered to be low (Fan et al., 2023; Rawson et al., 2020; Singh et al., 2021). Interestingly, the implementation of contact restrictions during the COVID-19 pandemic led to a decrease in infections in general, and a reduction of antibiotic usage and hospitalizations for non-COVID-19 infections were observed at the population level (Bürke et al., 2024; Nakitanda et al., 2022; Nymand et al., 2022).

COVID-19 may lead to a prolonged disease manifestation, commonly referred to as long-COVID or post-COVID-19 condition (PCC) (Ely et al., 2024), which impacts approximately 5% of individuals initially infected. The most frequently reported symptoms include exercise-induced dyspnea, fatigue, and cognitive difficulties (Wulf Hanson et al., 2022). The principal risk factor for a prolonged disease trajectory is the severity of the initial infection. Those who experience more severe initial infections are more likely to develop enduring symptoms. Non-hospitalized individuals typically report long-COVID symptoms lasting an average of 4 months, whereas hospitalized patients generally experience symptoms for an average of 9 months. Generally, the long-term prognosis for long-COVID is moderately good since an average of only 15% of individuals who are symptomatic three months after the infection exhibit symptoms 12 months post-infection. (C. Chen et al., 2022; Mao et al., 2020; Wulf Hanson et al., 2022)

### 2.4.2 Clinical features in children

Children infected with SARS-CoV-2 generally demonstrate a milder clinical presentation in comparison to adults (Cloete et al., 2022; X. Lu et al., 2020). Similar to adults, children primarily exhibit symptoms associated with respiratory tract infections, including cough, sore throat, rhinorrhea, and fever. However, a wide spectrum of symptoms has been documented (Cloete et al., 2022). Asymptomatic cases are more commonly observed in children compared to adults (Wang et al., 2023; Yu et al., 2022). The severe manifestations of COVID-19 are predominantly observed in children with underlying medical conditions and in younger children under the age of four. Children without predisposing medical conditions rarely

experience severe disease progression and those who do typically recover well. (Cloete et al., 2022; Dong et al., 2020; X. Lu et al., 2020)

The emergence of SARS-CoV-2 variants has led to significant changes in clinical characteristics, transmission patterns, and susceptibility to SARS-CoV-2 infection among children. In the case of the wild-type variant, children exhibited a lower susceptibility to infection compared to adults, with a risk ratio (RR) of 0.65. However, this difference diminished following the emergence of subsequent variants. (F. Chen et al., 2022; Chun et al., 2022) The Omicron variant in children exhibits a disease course similar to that of adults, characterized by increased transmissibility and a reduction in disease severity (Esper et al., 2023; Lewnard et al., 2022; Madewell et al., 2022; Menni et al., 2022).

## 2.5 Diagnostics

Diagnostic testing is essential for reducing the transmission of SARS-CoV-2. The demand for testing soared when the COVID-19 pandemic began, consequently leading to a global shortage of essential testing supplies. Of molecular tests that detect the virus RNA, the reverse transcription polymerase chain reaction (RT-PCR) test, performed preferably from nasopharyngeal specimens, is recognized as the gold standard for diagnosing SARS-CoV-2. (Hadaya et al., 2020; WHO, 2020c) Alternative testing methods include rapid antigen tests and serological assays.

### 2.5.1 Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR is a primary diagnostic tool for the identification of RNA viruses, including SARS-CoV-2 (Gröndahl et al., 1999; Hadaya et al., 2020). The first laboratory-developed test for COVID-19 to receive a recommendation from WHO was introduced on January 23rd, 2020, and was based on the detection of the SARS-CoV-2 E, RdRP, and N genes (Corman et al., 2020). The development of the test was facilitated by the rapid identification of the SARS-CoV-2 genetic structure (P. Zhou et al., 2020; Zhu et al., 2020). Since then, numerous laboratory-developed and commercial methods with different target genes have emerged for SARS-CoV-2 diagnostics.

The working principle of RT-PCR is summarized in **Figure 6.** First, a sample containing the pathogen's RNA is collected. The most commonly used sample type is a nasopharyngeal specimen (NPS) obtained with a nasopharyngeal swab (WHO, 2020c). Alternatively, swabbing can be processed with an anterior nasal swab or with a mid-turbinate swab. Both of the alternative swabbing methods have shown comparable efficacy during the early symptomatic days, though they might be less

sensitive for low-positive cases. (Gadenstaetter et al., 2021) In addition to swabs, saliva and sputum samples are also applicable (Poukka et al., 2021; Wyllie et al., 2020). However, slightly lower viral loads have been observed in comparison to NPS, and some individuals may struggle to produce enough saliva or an adequate sputum sample (Migueres et al., 2020; Uršič et al., 2022; Williams, Bond, Zhang, et al., 2020).

Upon collection, the specimens are transported to the analytical laboratory in either dry tubes or tubes containing a viral transport medium. The RNA in samples is converted into complementary DNA (cDNA) using an enzyme called reverse transcriptase. Following conversion, cDNA is amplified using PCR. During the process, pathogen-specific primers attach to the target cDNA, selecting the specific region for amplification through repeated cycles of heating and cooling. After amplification, the resulting DNA is detected using fluorescent dyes which emit fluorescence upon binding to DNA. The intensity of fluorescence correlates with the amount of amplified DNA, enabling the quantification of the target DNA. The cycle number at which the fluorescence exceeds a certain threshold is called the cycle threshold (Ct) value. A lower Ct value signifies a higher quantity of target RNA, as the DNA reaches the detectable level faster. (Bustin, 2000; Yang & Rothman, 2004)

In terms of viral diagnostics, RT-PCR presents several advantages. The method's ability to exponentially amplify the target gene results in superior analytical sensitivity (Yang & Rothman, 2004). Additionally, RT-PCR can be easily adapted to detect novel emerging pathogens (Corman et al., 2020). It also enables the analysis of high numbers of samples relatively fast. Alongside these benefits, the technique exhibits high specificity, and the semi-quantitative results provide valuable insights for clinicians (Yang & Rothman, 2004). However, certain challenges arise, such as the potential for false positives due to contamination or setting the cut-off for the Ct value too high (C. Y. T. Wang et al., 2020). Moreover, conducting point-of-care RT-PCR tests at ICU necessitates precise coordination from local laboratories (Möckel et al., 2022).

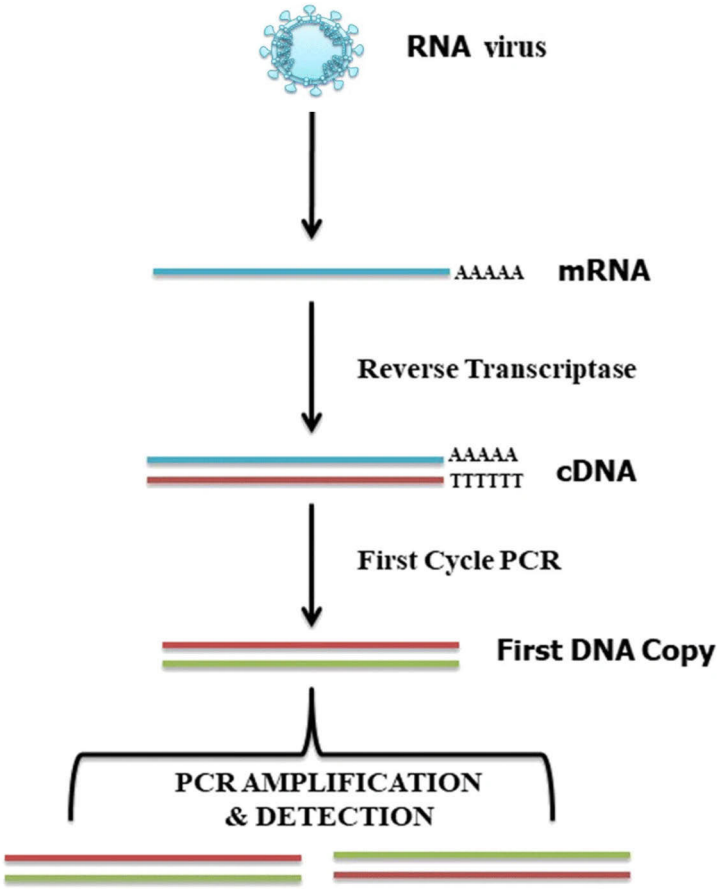


**Figure 6.** The operational principle of RT-PCR. First, a specimen containing the pathogen's RNA is collected and then converted into complementary DNA (cDNA) using reverse transcriptase. Following that, the cDNA is amplified through PCR and the resulting DNA is detected with fluorescence. Modified and reproduced from Jamshaid et al. Journal of the American Association of Pharmaceutical Scientists 2020, with the permission of the copyright holders.

### 2.5.2 Rapid antigen tests

Rapid antigen tests are convenient to use outside laboratories (Pilarowski et al., 2021). These tests have gained widespread adoption as commercial home testing kits and point-of-care diagnostic tools throughout the COVID-19 pandemic. Their benefits include user-friendliness and rapid results. Nevertheless, these tests have exhibited reduced sensitivity compared to RT-PCR. (Chaimayo et al., 2020; Lambert-Niclot et al., 2020; Peeling et al., 2021; Pilarowski et al., 2021; Porte et al., 2020; Weitzel et al., 2021)

The operation principle of the lateral flow rapid antigen test is summarized in **Figure 7**. Initially, a sample is obtained, typically with nasal or nasopharyngeal swab. Alternatively, certain commercial kits also offer the option for saliva collection. Subsequently, the sample is introduced into a buffer solution formulated to isolate viral antigens from the sample matrix. The prepared solution is then transferred to a test strip, which contains antibodies labeled with colored particles. These antibodies are specifically designed to bind to viral antigens. In the presence of viral antigens, the resulting antigen-antibody complexes attach to the test line, creating a visible colored line that indicates a positive test result. (Chaimayo et al., 2020)

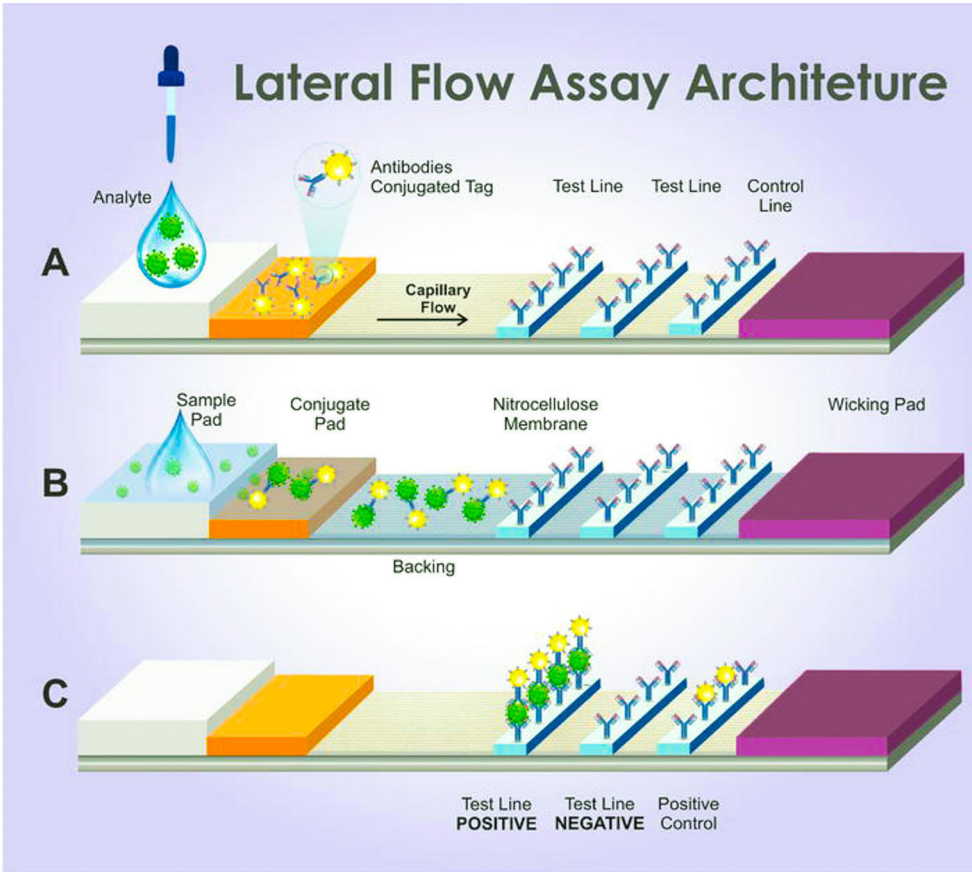


**Figure 7**. The working principle of lateral flow rapid antigen test. Viral antigens in the sample pass through the nitrocellulose membrane via capillary action (A). Labeled antibodies bind to antigens (B). The resulting antibody-antigen complexes interact with test line antibodies, while unbound labeled antibodies interact with control line antibodies (C). Reproduced from Costa et al. Current Topics and Emerging Issues in Malaria Elimination, 2021, with permission from the copyright holders.

### 2.5.3 Serology

Serological tests have an important role in assessing past COVID-19 infections, as most individuals develop a measurable immunoglobulin (Ig) response to SARS-CoV-2 (Bergeri et al., 2022; Gudbjartsson et al., 2020; Offergeld et al., 2023). However, serological assays are not applicable for diagnosing acute infection, since the serological IgM response typically appears 7-14 days following the onset of symptoms, while IgG responses are detectable starting from two weeks after infection onset and can prevail for up to 20 months (Alejo et al., 2022; Gudbjartsson et al., 2020; Hasan et al., 2021; Movsisyan et al., 2024). In addition to evaluating previous COVID-19 infections, serological testing can be utilized to assess COVID-19 vaccinations, which stimulate the production of antibodies specific to the SARS-CoV-2 S protein (Karachaliou et al., 2022). The relationship between serological response and long-COVID has also been investigated. However, findings indicate that no significant connection exists (Binswanger et al., 2024; Matta et al., 2022).

The limitations of serological methods in diagnosing acute infections have resulted in their primary utilization within research and epidemiological surveys (Offergeld et al., 2023). In such studies, evaluating prior COVID-19 infections through serology presents a few challenges. While IgG antibodies may persist for extended periods, they typically start to decline within two to four months post-infection, and individuals exhibiting mild serological responses may become seronegative within a few months from infection (Movsisyan et al., 2024). Furthermore, considerable variation in analytical performance among different test types occurs, and the antibody responses generated by vaccination complicate the interpretation of assays that analyze responses to the S protein (Jalkanen et al., 2021; Jääskeläinen et al., 2020; Karachaliou et al., 2022).

## 2.6 Treatment

Various pharmaceutical interventions, including SARS-CoV-2-specific antiviral drugs, anti-inflammatory agents, and monoclonal antibodies, have been thoroughly researched in numerous studies since the onset of the pandemic. Despite these extensive research efforts, only a few treatments have successfully progressed through large-scale clinical trials (WHO, 2022). Many treatment options that initially exhibited potential have ultimately failed to demonstrate effectiveness in real-world settings. For instance, both ivermectin and hydroxychloroquine have been shown to be ineffective in the treatment of COVID-19 (Alexandre B. Cavalcanti, 2020; López-Medina et al., 2021). The medications that are currently recommended (December 2024) are summarized in **Figure 8**.

As of December 2024, three medications are recommended by WHO to treat non-severe COVID-19. For patients at high risk for hospitalization (severely

immunosuppressed by immunodeficiency syndromes or immunosuppressants), the combination of nirmatrelvir and ritonavir is efficient in reducing hospitalization and mortality and is consequently strongly advised by WHO (Hammond et al., 2022). For those who cannot use nirmatrelvir-ritonavir, remdesivir or molnupiravir may be considered. However, for patients at moderate risk for hospitalization (over 65 years, significant comorbidities, lack of effective vaccinations), only nirmatrelvir-ritonavir is recommended. Nirmatrelvir-ritonavir should be initiated within five days of the onset of symptoms for optimal effectiveness. Consequently, its use in the treatment of severe COVID-19 is limited. (WHO, 2022)

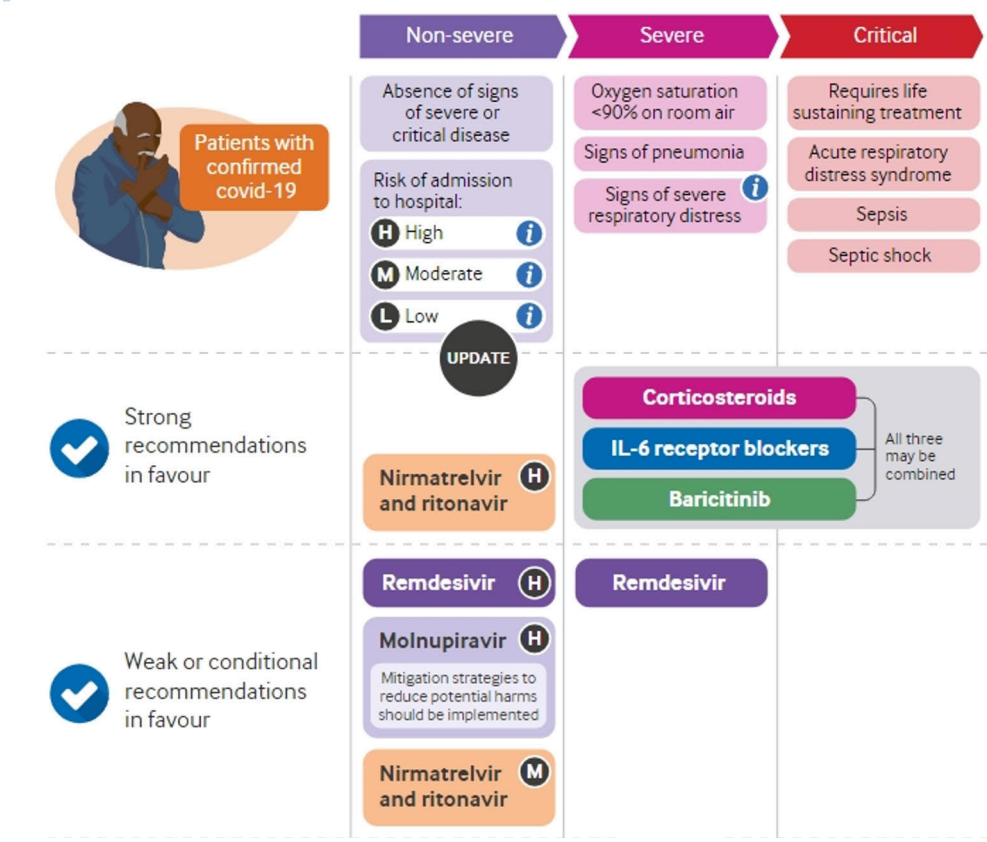


**Figure 8.** WHO living guidelines on medications for COVID-19, visual summary of recommendations. Nirmatrelvir-ritonavir is strongly recommended for high-risk patients with non-severe COVID-19. Corticosteroids, IL-6 receptor blockers, and baricitinib are strongly recommended for severe COVID-19. Molnupiravir is conditionally recommended for high-risk patients with non-severe disease, and remdesivir is weakly recommended for severe disease and for high-risk patients with non-severe disease. Modified and reproduced from Agarwal et al. BMJ 2020, with the permission of the copyright holders.

For the treatment of severe COVID-19, oral corticosteroids, interleukin-6 (IL-6) receptor blockers (tocilizumab or sarilumab), and Janus kinase (JAK) inhibitors (baricitinib) have been proven efficient, and are recommended by WHO (Abani et al., 2021; Lin et al., 2022; RECOVERY-Collaborative-Group, 2021). Combination therapies incorporating these three pharmaceuticals are also feasible. (WHO, 2022) Additionally, while remdesivir can be used, its effectiveness is likely limited (Hongchao Pan et al., 2022).

In addition to pharmaceutical solutions, supportive care is crucial in the management of COVID-19. Principal modalities of supportive treatment include fluid replacement and oxygen therapy (Chen et al., 2020; T. Wang et al., 2020; Ziehr et al., 2020). Oxygen therapy aims to maintain adequate oxygen levels in the vascular system during various degrees of respiratory failure. Oxygen supplementation can be adjusted according to the severity of the respiratory disorder. For milder respiratory issues, a simple nasal cannula provides enough oxygen, while more serious cases may require high-flow oxygen therapy or non-invasive mechanical ventilation. In critical cases, patients may need invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO) treatment. (Chen et al., 2020; Huang et al., 2020; Ziehr et al., 2020) Additionally, the implementation of the prone position has demonstrated advantages for patients undergoing mechanical ventilation (Mathews et al., 2021; Ziehr et al., 2020).

## 2.7 Prevention

The methods of prevention can be roughly categorized into non-pharmaceutical and pharmaceutical interventions. The latter mainly focuses on COVID-19 vaccinations. While the combination of two monoclonal antibodies, tixagevimab-cilgavimab, was used pre-Omicron for preventive purposes in special circumstances, particularly for individuals who are severely immuno-compromised (Akinosoglou et al., 2022; WHO, 2023b), most COVID-19 treatments have not shown efficacy in preventing the transmission (Hammond et al., 2024).

### 2.7.1 Non-pharmaceutical interventions

Non-pharmaceutical interventions refer to measures implemented to reduce the transmission of pathogens across populations without using pharmaceutical methods. These methods were widely used during the COVID-19 pandemic (Wong et al., 2020). The strategies employed ranged from subtle suggestions, such as recommendations to increase hand washing, to more significant measures like mandatory face mask usage in public locations. At the other end of the spectrum were strict restrictions, including lockdowns, quarantines, and limits on gatherings

(Talic et al., 2021). The most applicable methods have minimal negative impacts on individuals while providing a significant reduction in transmission.

The impact of the non-pharmaceutical intervention also depends on the pathogen. For instance, the pathogens that are mainly transmitted by direct or indirect contact such as norovirus, are more efficiently reduced by improvements in hand hygiene (Assab & Temime, 2016). Although the implementation of increased hand washing is relatively mild in terms of social impact, its effectiveness in mitigating the transmission of SARS-CoV-2 is likely limited as SARS-CoV-2 is primarily transmitted through respiratory droplets and aerosols (Harrison et al., 2020). However, a systematic review by Talic et al. indicates that hand washing reduces SARS-CoV-2 infection risk by 53%. Nevertheless, it should be noted that all three included studies had at least a moderate risk for bias, and hand hygiene non-compliance may also correlate with neglecting other hygiene practices (Talic et al., 2021). In theory, the use of face masks and social distancing beyond two meters should significantly reduce the SARS-CoV-2 transmission as respiratory droplets can rarely travel beyond two meters, and masks can further inhibit the dispersal of these droplets (Jarvis, 2020). Talic et al. reviewed mask usage from six studies and found a reduction rate of 53%, with five of these six studies showing a significant reduction in transmission by mask-wearing (Talic et al., 2021). Wearing face masks at home during COVID-19 infection reduced the rate of secondary transmission in comparison with individuals who did not wear face masks (SAR reduction from 69% to 40%) (Baker et al., 2022). However, the type of mask is a critical factor, with FFP3 masks offering more protection than commonly used surgical face masks to both the wearer and those in proximity (Wise, 2021).

Stricter non-pharmaceutical interventions such as isolations, lockdowns, border closures, school closures, and restrictions on gatherings can substantially reduce transmission, but they may also lead to long-lasting negative consequences (Kauhanen et al., 2023; Talic et al., 2021). For instance, the reports on the effect of school closures are contradictory, with other studies reporting no effect on SARS-CoV-2 transmission (Iwata et al., 2020) while other studies reported significant reductions in transmission (Auger et al., 2020). The studies on such interventions are vulnerable to bias, as several public interventions were often implemented simultaneously. Nevertheless, strict measures have shown significant reductions in transmission in studies: Quarantine implementation reduced the basic reproduction number by 37% to 88% (Nussbaumer-Streit et al., 2020), massive lockdown in Italy reduced R0 values from 2.03 to 0.76 in three weeks (Guzzetta et al., 2021), and social isolation within household members led to 18-fold decrease in transmissibility (Y. Wang et al., 2020). However, the overall impact of interventions is highly dependent upon the prevalence of SARS-CoV-2 in the population and the effectiveness with which the implemented restrictions are executed in real life (Talic et al., 2021).

### 2.7.2 Vaccines

The rapid development of COVID-19 vaccines was unprecedented and demonstrates a significant achievement in medical science. This achievement can be attributed to three key factors: First, the extensive prior knowledge of coronaviruses, particularly related to SARS-CoV-1 and MERS-CoV; second, the advancement of novel vaccine platforms, including adenovirus vectors and messenger RNA (mRNA) technology; and third, successful global collaboration.

Vaccine development efforts concentrated on the spike protein of SARS-CoV-2 due to its significant similarity to the S protein of SARS-CoV-1 (Lan et al., 2020). This strategy drew from previous research on coronaviruses, which indicated that the S protein of SARS-CoV-1 was a promising vaccine target (Du et al., 2009). Additionally, antibodies produced against this protein were found to be enduring and effective in triggering a robust immune response (Cao et al., 2010). Furthermore, several S protein-based vaccines on SARS-CoV-1 and MERS-CoV had been demonstrated effective in preclinical studies and animal models (Gao et al., 2003; Kim et al., 2014; Li et al., 2013).

The swift identification of the SARS-CoV-2 structure in January 2020 facilitated the start of vaccine development, leading to the initiation of the first clinical trials in March 2020 (Jackson et al., 2020; Polack et al., 2020; Voysey et al., 2021; Zhu et al., 2020). While the development of various types of vaccines was initiated, mRNA and adenovirus vector vaccines emerged as the most successful candidates. The progression of adenovirus vector vaccines was significantly boosted by prior promising findings from animal models related to MERS-CoV and SARS-CoV-1, as well as from clinical trials involving influenza A and Ebola (Coughlan et al., 2018; Gao et al., 2003; Gilbert & Warimwe, 2017; Kim et al., 2014). The first adenovirus vector vaccine was authorized for use in December 2020 and was reported safe and efficient against SARS-CoV-2, especially against severe disease (Voysey et al., 2021). However, a few months after the launch of adenovirus vector vaccines, an increased risk of vaccine-induced immune thrombotic thrombocytopenia was reported. This very rare yet potentially dangerous adverse effect led to a decline in the use of adenovirus vector vaccines and further solidified the dominance of mRNA vaccines. (Cines & Bussel, 2021).

The foundation of mRNA vaccine development was built on over 30 years of active mRNA research (Karikó et al., 2005; Malone et al., 1989). The operating principle of the mRNA vaccine is illustrated in **Figure 9.** In brief, messenger RNA that encodes viral protein is enclosed in a lipid envelope, which delivers the mRNA to human cells that produce components of the virus recognized by immune cells. Prior to the COVID-19 pandemic, mRNA vaccines had been explored in various clinical and pre-clinical trials targeting viruses such as influenza A and B viruses, zikavirus, HIV, RSV, ebolavirus, and rabies virus. However, none of these vaccines

had been widely implemented before the onset of the pandemic. (Chaudhary et al., 2021; Pardi et al., 2018)

The mRNA vaccines developed against COVID-19 demonstrated remarkable efficacy following two doses, achieving efficacy rates of 95% for BNT162b2 developed by Pfizer-BioNTech and 94% for mRNA-1273 produced by Moderna. Additionally, both vaccines demonstrated a favourable safety profile, reinforcing their status as leading global vaccines. (Baden et al., 2021; Polack et al., 2020) The waning of vaccine or infection-induced immunity was reported, leading to the recommendation for additional booster doses a few months after the initial two doses (Andrews, Tessier, et al., 2022). The emergence of SARS-CoV-2 variants further decreased the vaccination efficacy (Chapter 2.3.2). Although efficacy on preventing infection substantially decreased from the Alpha to Omicron variant, the efficacy against hospitalization remained considerably high (78%-92% at >4 months after the last dose), and the distribution of booster doses continued to post-pandemic era for those at risk of severe disease (Andrews, Tessier, et al., 2022; Ferdinands et al., 2022; Madewell et al., 2022). A study by Watson et al. estimated that COVID-19 vaccinations averted approximately 20 million deaths from COVID-19 during the first year since implementation (Watson et al., 2022). In Finland, Finnish Institute for Health and Welfare (THL) estimated that 7300 COVID-19 fatalities were averted by vaccinations (THL, 2023).

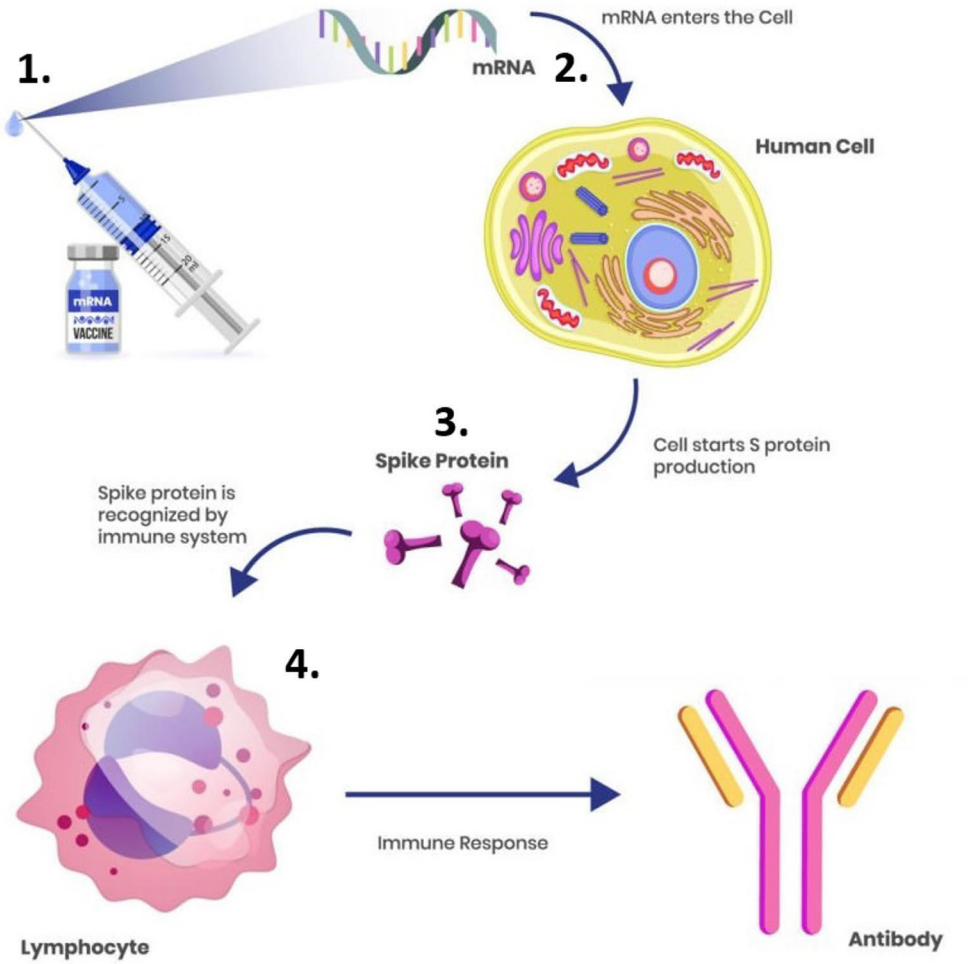


**Figure 9.** Working principle of mRNA vaccination. 1. Messenger RNA (mRNA) that encodes the production of viral spike (S) protein is enclosed in a lipid envelope and injected into humans. 2 Lipid envelope fuses with human cells, delivering the mRNA to human cells 3. Human cells produce the S protein of the virus 4. The S protein is recognized by immune cells, and an immune response develops. Figure modified from istockphotos.com.

# 3 Aims

This doctoral thesis was conducted to evaluate novel specimen collection methods for the laboratory diagnosis of SARS-CoV-2 and to examine the household transmission of SARS-CoV-2-positive and -negative respiratory infections.

The specific aims of the present study were:

1. To compare the diagnostic efficacy and tolerability of molded plastic nasopharyngeal swabs (Finswab) with routinely used flocked nylon nasopharyngeal swabs for respiratory virus detection.
2. To compare the diagnostic performance and tolerability of saliva versus nasopharyngeal swab collection in SARS-CoV-2 detection.
3. To examine the role of children in the household transmission of SARS-CoV-2-positive and -negative infections.
4. To investigate the household transmission of SARS-CoV-2 with serological assays in addition to RT-PCR.

# 4 Materials and Methods

The present thesis comprises four original studies. A summary of the studies' methodology, and main outcomes are presented in **Table 2**.

**Table 2**. Summary of participants, conduct and outcomes in studies I-IV

| Original Study | Participants | Study Conduct | Main Outcomes |
|---|---|---|---|
| Study I (Finswab Trial) | 112 participants, ≥ 18 years | Nasopharyngeal sampling with the study swab (Finswab) and a reference swab. | Human β-actin $C_T$ values, rhinovirus $C_T$ values, discomfort values |
| Study II (Saliva Study) | 250 participants, ≥ 10 years | Saliva sampling after nasopharyngeal specimen collection. | Saliva sensitivity and specificity, SARS-CoV-2 detection rate, SARS-CoV-2 $C_T$ values, discomfort values |
| Study III (Household transmission of Sars-Cov-2-positive and -negative infections | 700 participants (175 children aged 11-13 years and their household members) | Weekly questionnaires on respiratory symptoms, SARS-CoV-2 RT-PCR or antigen tests of symptomatic or exposed participants. | SAR of SARS-CoV-2-positive and -negative infections, clinical features |
| Study IV (Household transmission and Serology) | 700 participants (175 children aged 11-13 years and their household members) | Seroprevalence at three time points, seroconversion five weeks after SARS-CoV-2 infection in the household. | SARS-CoV-2 seroprevalence, SAR based on seroconversion, sensitivity and specificity of serology versus RT-PCR |

Abbreviations: $C_T$, cycle threshold; SAR, secondary attack rate

## 4.1 Study design, setting, and subjects

### 4.1.1 Study I

In study I, nasopharyngeal specimens were collected with the study-specific swab (FinSwab) and reference swab. Attending nurses collected the swab specimens from the nasopharynx at a COVID-19 drive-in testing station of the Turku University

Hospital, Turku, Finland, during October 2020 and February 2021. The eligibility criteria included age ≥18 years and need for a SARS-CoV-2 PCR test due to symptoms indicative of COVID-19. No exclusion criteria were applied. Ultimately, 115 participants were recruited.

### 4.1.2 Study II

In study II, saliva and nasopharyngeal specimens were subsequently collected at a COVID-19 drive-in testing station of the Turku University Hospital, Turku, Finland, during March and April 2022. Nasopharyngeal swab specimens were obtained by attending nursing staff, while the collection of saliva samples was conducted under the guidance of study nurses. The eligibility criteria included age ≥10 years, a need for a SARS-CoV-2 PCR test, and having a fluent understanding and ability to communicate in Finnish. The exclusion criteria were previous participation in the current study. The population attending the SARS-CoV-2 testing primarily comprised healthcare professionals and individuals considered to have elevated risk for severe COVID-19, in accordance with Finnish guidelines on SARS-CoV-2 testing at the time. Ultimately, 250 participants were recruited.

### 4.1.3 Study III-IV

Studies III and IV were conducted within the prospective birth-cohort study, the STEPS study (Steps to the Healthy Development and Well-being of Children). In the STEPS Study, 1805 children born in 2008 to 2010 in the Hospital District of Southwest Finland are systematically followed from pregnancy to early adulthood. The recruitment of participants to studies III and IV was conducted among families with children participating in the STEPS study. A total of 175 children aged between 11 and 13 years, along with their household members, a total of 700 participants, volunteered to participate in the substudy between June and August 2020.

Participants were closely monitored for daily symptoms of respiratory infections, as well as for SARS-CoV-2 PCR and home antigen testing results, and the treatment of respiratory infections. Daily symptoms were monitored by weekly questionnaires from June 2020 until the study concluded in April 2022 using REDCap. (Harris et al., 2019; Harris et al., 2009). The questionnaires included information on all household members. Households were asked to record the dates of symptoms of respiratory infections, such as fever, cough, runny nose, sore throat, fatigue, shortness of breath, headache, loss of smell or taste, muscle pain, diarrhea, vomiting, and any other related symptoms. Participants were also asked to document physician visits and any diagnoses and treatments received. Additionally, households noted the dates and results of nasopharyngeal specimens tested for SARS-CoV-2 PCR in a

laboratory and home antigen test outcomes for each member of the household. Beyond weekly questionnaires, data on SARS-CoV-2 PCR test results, related hospital admissions, and medications were gathered from the electronic medical records of the Hospital District of Southwest Finland. Information regarding COVID-19 vaccinations for participants was collected through separate questionnaires. Data on emerging SARS-CoV-2 variants in Southwest Finland were sourced from routine surveillance conducted by the Department of Clinical Microbiology at Turku University Hospital.

## 4.2 Specimen collection

### 4.2.1 Nasopharyngeal swab (NPS)

In all studies, NPS specimens were collected by attending nurses at COVID-19 testing sites according to the guidelines set by the hospital district.

In study I, a nasopharyngeal specimen was first obtained using a reference swab (Copan flocked nylon swab [FLOQSwab] model 503CS01 or model XA5S108B01), followed by a sample taken with the FinSwab from the same nostril. The participants assessed for discomfort (n=36) were sampled in randomized order. The same nostril was sampled to minimize any potential bias that could arise from the anatomical variations of the nostrils. To evaluate the impact of the transport medium on diagnostic accuracy, swabs were collected in both dry transport tubes (n=48) and tubes containing Xpert viral transport medium (n=28; VTM; Cepheid, USA). The swabs collected in dry tubes were then suspended in 1 ml of phosphate-buffered saline (PBS), vortexed, and allowed to settle for 10 minutes.

In study II, nasopharyngeal specimens were obtained with a Bioer Sample Collection Kit (transport tube with 2 mL of sample preservative fluid; Hangzhou Bioer Technology, Hangzhou, China). The collection process followed the guidelines provided by the Turku University Hospital and was conducted by the attending nurses.

In studies III and IV, all participants displaying symptoms indicative of SARS-CoV-2 infection, as well as those who had been exposed to the virus, were directed to undergo NPS specimen collection. If a study participant received a positive test result for SARS-CoV-2, all other household members were referred for NPS specimen collection for SARS-CoV-2 PCR testing, either on the same day or the subsequent day. All SARS-CoV-2 PCR-negative participants were additionally tested one week after the initial test. If any household member developed respiratory symptoms later, the affected participant was required to undergo an additional SARS-CoV-2 PCR test at the onset of those symptoms.

#### 4.2.1.1 Novel NPS design (Study I)

The initial prototypes of the novel NPS were developed by 3D printing technology. These prototypes underwent a series of evaluations, including in vitro testing and a pilot study (conducted by the manufacturer) assessing functionality and discomfort in 10 healthy adult volunteers. Ultimately, the final design, referred to as the FinSwab, was selected for production. This final product was manufactured using a plastic injection molding technique. Both the prototypes and the final products were manufactured by Valukumpu Oy, a company based in Finland. The prototypes, along with the Finswab, are presented in **Figure 10**.

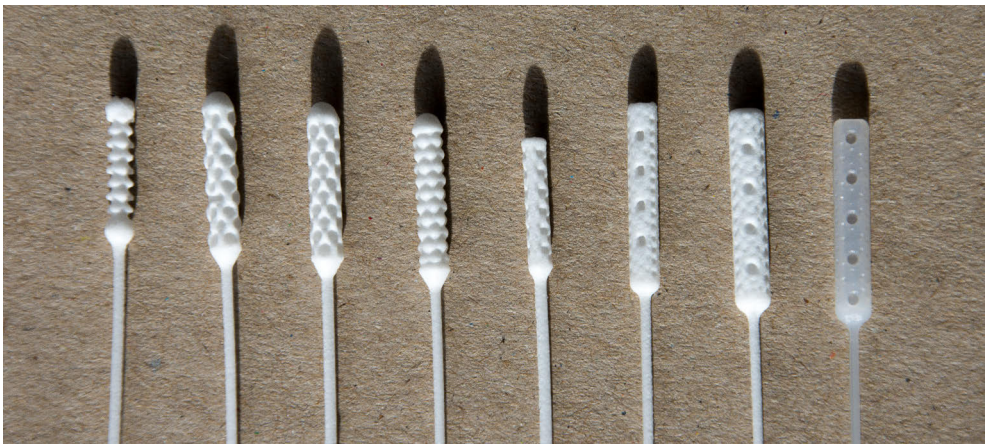

**Figure 10.** The 3D-printed prototypes and the final plastic injection molded product (Finswab, at the right edge). Photography and editing by Jaakko Ahti.

### 4.2.2 Saliva (Study II)

Saliva specimens were obtained with a DNA/RNA Shield Saliva/Sputum Collection Kit (Zymo Research, USA), in accordance with the kit's instructions. Prior to saliva collection, detailed inquiries were made regarding the presence of symptoms and their duration, as well as any recent activities including eating, drinking, smoking, using mouthwash, or brushing teeth within the thirty minutes preceding the collection. The date and time of saliva collection were recorded.

Preceding saliva sampling, individuals were instructed to rinse their mouths with water. The study nurse subsequently guided the participants on the procedure for collecting a saliva specimen via the passive drooling method. The target volume for the saliva specimen was established at 2 mL, which was then combined with 2 mL of DNA/RNA shield buffer and inverted firmly ten times. In instances where a participant was able to produce less than 2 mL of saliva, DNA/RNA shield buffer was added in a 1:1 ratio relative to the volume of saliva collected.

### 4.2.3 Discomfort evaluation (Study I-II)

In study I, the discomfort associated with nasopharyngeal sampling was assessed among 36 participants. Each participant underwent collection of a reference swab (Copan FLOQSwab model 503CS01) and a FinSwab from the same nostril in a randomized order. Participants were instructed to close their eyes during the sample collection process. After both samples were placed into transport tubes, participants were permitted to open their eyes. Subsequently, they were asked to evaluate the discomfort experienced during the swabbing on a scale from one (no discomfort) to five (extreme discomfort). Following this, the study nurse inquired about the participants' preference of swab for future nasopharyngeal sampling.

In the saliva study (II), the discomfort evaluation was assessed among all 250 participants. Following the collection of both specimens, the participants were requested to evaluate the discomfort associated with each sampling method on a scale from one (no discomfort) to five (extreme discomfort). Subsequently, the preferred method for future COVID-19 testing was inquired.

## 4.3 Laboratory methods

### 4.3.1 RT-PCR

In all studies, NPS specimens were analyzed by a laboratory-developed quantitative real-time PCR (RT-qPCR) developed for the detection of SARS-CoV-2 at the Department of Clinical Microbiology, Turku University Hospital. In studies III and IV, a few samples were reported to have been collected and analyzed in private COVID-19 testing stations, or outside the Southwest Finland region.

In study I, specimens were analyzed for SARS-CoV-2 using PCR immediately following collection. Specimens were then stored at −80°C for further assessment with a multiplex RT-PCR assay for respiratory viruses. In the second study (II), specimens were gathered in groups of 90 specimens. The NPS specimens were stored at +4°C, while saliva specimens were stored at room temperature in accordance with the guidelines provided by the specimen collection kit, for a maximum duration of 16 days. Before the extraction process, the saliva samples were subjected to heating at 37°C for 10 minutes.

A laboratory-developed multiplex RT-qPCR assay for SARS-CoV-2 was performed employing primers and probes recommended by the WHO. In all studies (I-IV), the E gene was utilized as the main diagnostic target, while the S gene - with or without the H69-V70 deletion - was used to distinguish the circulating variants of SARS-CoV-2 in studies II-IV. The RT-qPCR was performed in 25 µL reactions, incorporating 5 µL (study I) or 9 µL (study II) of nucleic acid. Bioline SensiFAST

probe one-step master mix (Meridian Bioscience, USA), and a Mic qPCR cycler (Bio Molecular Systems, Australia) were utilized. The interpretation of cycle threshold ($C_T$) results for the E gene channel was as follows: $C_T < 38$, positive; $C_T$ 38 to 39.9, low positive; $C_T \geq 40$, negative. The $C_T$ results from the S gene channel were interpreted as follows: $C_T < 42$, positive; $C_T \geq 42$, negative.

In study I, a multiplex RT-qPCR assay (Allplex respiratory panels 1 to 3; Seegene, Republic of Korea) was conducted following the manufacturer's instructions using a Bio-Rad CFX96 instrument to detect 16 respiratory viruses. The viruses included rhinovirus, adenovirus, enteroviruses, human coronaviruses 229E, NL63, and OC43, human metapneumovirus, human bocavirus, influenza A and B viruses, parainfluenza virus types 1, 2, 3, and 4, and respiratory syncytial viruses A and B. $C_T$ results were interpreted as follows: $C_T < 37$, positive; $C_T$ 37 to 42, weakly positive; $C_T > 42$, negative.

In study I, RT-qPCR for human β-actin mRNA was conducted using Bioline SensiFAST probe one-step master mix (Meridian Bioscience, USA) and analyzed with a Rotor Gene 3000 (Qiagen, Germany).

In study II, in addition to laboratory-developed tests (LDT), two commercial SARS-CoV-2 RT-qPCR tests were employed utilizing a Bio-Rad CFX96 Real-Time Cycler. The tests included SARS-CoV-2 RT-qPCR Reagent Kit targeting the N and ORF1ab genes (Wallac Oy, PerkinElmer, Finland) and SARS-CoV-2 RT-qPCR Plus Reagent Kit (Wallac Oy, PerkinElmer, Finland) targeting the N, E, ORF1ab, and Sdel genes. The latter kit was designed for monitoring SARS-CoV-2 variants.

### 4.3.2 Rapid antigen testing (Study III-IV)

In studies III and IV, participants were referred to NPS sample collection by healthcare professionals according to the Finnish national guidelines in effect at the time. As of January 2022, the national guidelines were revised to permit the use of home antigen tests as an alternative to laboratory testing. While study participants continued to be encouraged to undergo nasopharyngeal specimen collection by healthcare personnel, the utilization of home antigen tests available at pharmacies and local stores were also permitted.

### 4.3.3 Serological assays (Study IV)

Serum samples for antibody analysis were collected from all participants at the time of recruitment and at 6-8 months and 12-15 months post-recruitment. Furthermore, if any household member tested positive for SARS-CoV-2 using PCR or a home antigen test, serum samples were collected from all household members five weeks following the initial infection.

Serological samples were analyzed using two distinct automated IgG assays to assess the specific responses to N and S proteins of SARS-CoV-2. For the analysis of anti-N-IgG, a chemiluminescent microparticle immunoassay (Abbott Alinity I SARS-CoV-2 IgG; Abbott, Illinois, USA) was employed. The results were interpreted following the manufacturer's guidelines: < 1.40 AU/ml, negative; ≥ 1.40 AU/ml, positive. For a sensitivity analysis, the cutoff value was set at +2 standard deviation of anti-N-IgG levels at recruitment, resulting in the following interpretation: <0.66 AU/ml, negative; ≥ 0.66 AU/ml, positive. Anti-S-IgG were measured using a chemiluminescent assay (CLIA) (Diasorin Liaison XL SARS-CoV-2 S1/S2 IgG; DiaSorin, Saluggia, Italy). The results were interpreted following the manufacturer's guidelines: < 12 AU/ml, negative; 12-15 AU/ml, indifferent; > 15 AU/ml, positive.

## 4.4 Transmission in households (Study III-IV)

### 4.4.1 Definitions and outcome measures

In studies III and IV, an acute respiratory infection was characterized by a sudden onset of symptoms including cough, runny nose, or sore throat, with or without a fever. Participants with two or more doses of the COVID-19 vaccine obtained were considered fully vaccinated. The COVID-19 vaccination was deemed effective starting 14 days after the date of vaccination. Participants were classified as adults or children based on whether they were parents or offspring, regardless of their actual ages (three of the children were over 18 years old).

In study III, an index case was the first household member to exhibit symptoms consistent with COVID-19 or another acute respiratory tract infection. A respiratory infection cluster was defined as including all respiratory infections within a household that occurred within a 14-day period following the onset of symptoms in the index case. A respiratory infection cluster was classified as SARS-CoV-2 positive if any member of the household tested positive for SARS-CoV-2. Conversely, a cluster was categorized as SARS-CoV-2 negative if at least one household member received a negative SARS-CoV-2 PCR test result during the current infection cluster, and none of the tests conducted were positive for SARS-CoV-2. All non-index household members who presented symptoms of infection during a 14-day cluster period were classified as secondary cases. Exclusions from the analysis were applied in specific situations. Entire clusters were excluded if the index person could not be identified or if no SARS-CoV-2 tests were conducted for any symptomatic member of the household during the cluster investigation. Additionally, within the SARS-CoV-2-positive clusters, individual infections were excluded if they were asymptomatic (n=7) or if symptomatic individuals had repeatedly negative SARS-CoV-2 PCR test results.

In study IV, the index case was the first household member who tested positive for SARS-CoV-2 via PCR or antigen test. If multiple household members received a positive test result on the same day, the index case was the first to exhibit symptoms. Other household members who tested positive for SARS-CoV-2 with a PCR test or developed an anti-N-IgG response were classified as secondary cases.

Transmission in households was assessed by the household secondary attack rate (SAR) as the primary outcome measure. SAR was calculated by dividing the number of secondary cases by the total number of non-index household members. In study III, SAR was determined based on the onset of respiratory symptoms appearing within 14 days following the index case's symptom onset. In study IV, SAR was identified through both PCR and anti-N-IgG antibodies. SARS-CoV-2 anti-N-IgG positive participants (20-60 days after the index case's PCR-positive infection) were categorized as secondary cases, alongside PCR-positive participants within 21 days of the index case.

## 4.5 Statistical analysis

The statistical analyses were performed using SPSS Statistics 26 (IBM, USA) in study I, JMP Pro version 16.2.0 (JMP, United Kingdom) in study II, and SAS version 9.4 (SAS Institute) in studies III and IV. The level of significance was set at $P < 0.05$ (2-tailed). Percentages were compared using a $\chi 2$ test, means using paired or independent two-sample t-test as applicable, and medians using the Mann-Whitney U test or Wilcoxon signed rank test.

In the study I, Pearson's correlation coefficient was used to analyze the correlation of $C_T$ values between FLOQSwab and FinSwab specimens from each patient. A $C_T$ value of 42.0 was given for negative results in instances where rhinovirus was detected with only one of the swabs. To assess the discomfort linked to the nasopharyngeal sampling, two swabs were taken from each participant in a random sequence. The randomization code was generated using SAS 9.4 for Windows (SAS Institute, USA) through a method of random permuted block randomization. This approach ensured that each type of swab had an equal number of instances as both the first and second nasopharyngeal swab.

In the study II, Cohen's kappa coefficient was calculated to assess the agreement of the results between the specimen types. Pearson's or Spearman's correlation coefficients were used to correlate the $C_T$ values, as applicable. In instances where the PCR test was positive from only one specimen type, a $C_T$ value of 40.0 was given for a negative LDT E gene, PerkinElmer N, and ORF1ab gene result, and a $C_T$ value of 42.0 was given for a negative LDT S gene and LDT and PerkinElmer Sdel result. The sensitivity and specificity (with a 95% confidence interval [CI]) of the saliva tests were calculated using the respective PCR test result from NPS as the reference

standard. The Cochran-Mantel-Haenszel test was used to evaluate the effect of eating, drinking, smoking, using mouthwash, and brushing teeth on the qualitative results between specimen types. The sample collection time was treated as a continuous variable and binary logistic regression was used to evaluate the association between the time of day of the saliva specimen collection and the test results from the saliva samples.

In the study III, mixed effects logistic regression was used to examine symptoms between SARS-CoV-2–positive and -negative infections. The test result was treated as a fixed effect, while participant and household identities were random effects. Repeated entries of individuals and households were considered. The duration of symptoms underwent a natural logarithmic transformation and was analyzed with a linear mixed model. The same effects used in the prevalence models were applied. The differences between children and adults were examined using the same tests, with the household role treated as a fixed effect.

In the study III, a linear mixed model was used to analyze the statistical differences between SARS-CoV-2–positive and SARS-CoV-2–negative infection clusters. The cluster's SARS-CoV-2 positivity was a fixed effect, while household identity was a random effect. Infection clusters were treated independently, and differences and characteristics were examined using a Wald risk difference test.

In the study IV, the relationships between categorical variables and anti-N-IgG and anti-S-IgG status were examined using Chi-square test and Fisher's exact test. Differences in continuous values of anti-N-IgG and anti-S-IgG across different groups were examined with Wilcoxon rank-sum test. The SARs among different groups were analyzed using Wald's risk difference test. Sensitivities and specificities were calculated to study anti-N-IgG seroconversion against PCR. Sensitivity analyses were performed in the same manner using a new cutoff value.

## 4.6 Ethics

All participants in the studies provided written informed consent prior to participating. In the saliva study (II), parents provided written consent for children under 15 years of age. In the household transmission studies (III-IV), adolescent participants over 12 years of age provided separate written consent, while parental written consent was obtained for all children under 18 years of age. The study protocols were approved by the Ethical Committee of the Hospital District of Southwest Finland (Study I: no. 21/1801/2020; Study II: no. 103/1801/2021; Studies III-IV: no. 20/1801/2020).

# 5 Results

## 5.1 Diagnostic efficacy and tolerability of molded plastic nasopharyngeal swab

A total of 115 individuals were recruited in the study evaluating molded plastic nasopharyngeal swab. After the specimen collection, three individuals refused to participate. Among the final 112 participants included in the study, 75 (67%) were female, and the mean age was 38 years (range 18 to 76, SD 14). The effect of transport tubes was assessed in a subset of 76 (68%) participants, and the discomfort was evaluated in a subset of 36 (32%) participants. The summary of the results is presented in **Table 3.**

**Table 3.** The main results on diagnostic efficacy and tolerability of Finswab in comparison to FLOQSwab.

| Outcome | Participants, No. | Finswab | FLOQswab | Comparison |
|---|---|---|---|---|
| Human β-actin ($C_T$), mean (SD) | 112 | 22.3 (3.61) | 22.1 (3.50) | P = 0.46 |
| Dry transport tube | 48 | 20.5 (2.93) | 20.0 (2.99) | P = 0.31 |
| Viral transport medium | 28 | 22.0 (2.35) | 23.0 (2.60) | P = 0.08 |
| Rhinovirus RNA ($C_T$), median (IQR) | 26 | 34.8 (28.6 to 41.0) | 35.0 (29.7 to 40.3) | P = 0.12 |
| Discomfort evaluation: swab preference, no. (%) | 36 | 23 (64%) | 12 (33%) | P = 0.06 |

The participants were sampled with Finswab and reference swab at a COVID-19 drive-in testing station. The individuals admitted to testing were not hospitalized and exhibited mild to moderate respiratory symptoms or other signs suggestive of SARS-CoV-2 infection.

### 5.1.1 Diagnostic efficacy

The efficacies of nasopharyngeal swabs were compared by examining human β-actin $C_T$ values, the number of virus-positive results, and the $C_T$ values of virus-positive results.

Human β-actin was found in all collected specimens in both nasopharyngeal swabs. The distribution of human β-actin $C_T$ values is shown in **Figure 11**. Mean human β-actin $C_T$ values were similar between FinSwab (22.3) and FLOQSwab (22.1; P = 0.46). In an analysis by transport tube, the differences in human β-actin mean $C_T$ values were not statistically significant between Finswab and FLOQSwab in dry tubes (20.5 and 20.0, respectively; P = 0.31) or in viral transport medium (VTM) (22.0 and 23.0, respectively; P = 0.08).

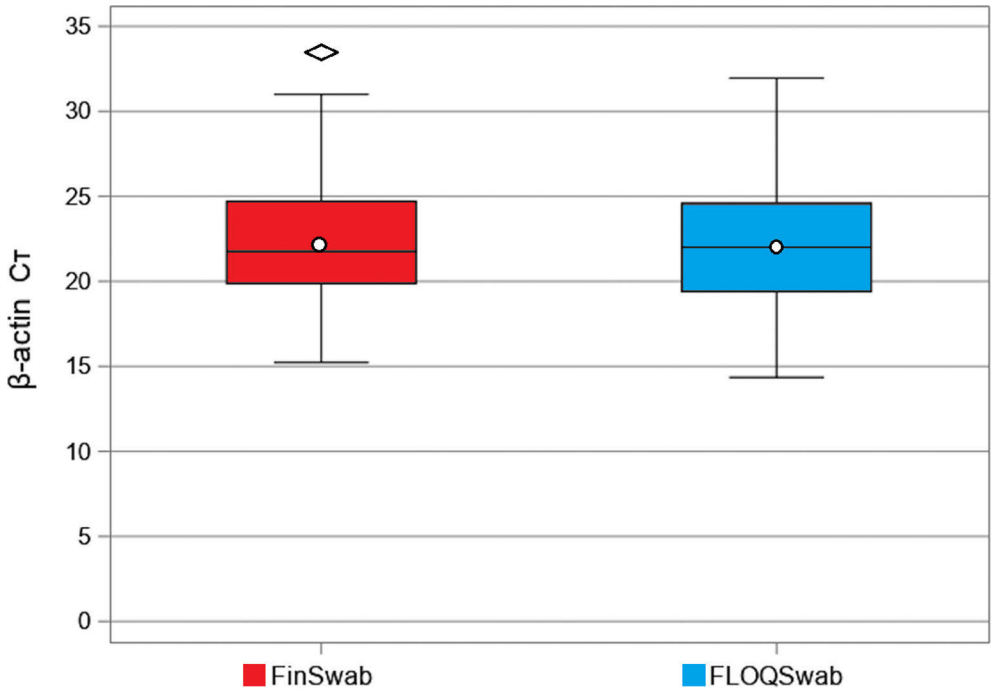


**Figure 11.** Human β-actin mRNA $C_T$ values from FinSwab and FLOQSwab. Medians, interquartile ranges, and minimum and maximum values are presented. The means are marked with a dot. An outlier is marked with a diamond. Reproduced and modified with the permission of the copyright holders from the original study I (CC BY 4.0).

A total of 30 individuals tested positive for examined viruses. Among virus-positive participants, 26 were rhinovirus-positive, four were SARS-CoV-2-positive, and one participant was positive for both rhinovirus and CoV-OC43. All SARS-CoV-2- and CoV-OC43-positive cases were found with both swabs. The mean

SARS-CoV-2 $C_T$ values were similar with Finswab (19.5; SD 3.90) and FLOQSwab (17.7; SD 3.96). The $C_T$ values for CoV-OC43 were 24.6 with Finswab and 26.2 with FLOQSwab.

Of the 26 rhinovirus-positive participants, 19 had positive results with both swabs. Among the seven discrepancies, three were only detected by Finswab and four only by FLOQSwab, with five of the discrepant results being low positives. Rhinovirus $C_T$ values showed similar results between Finswab (median 34,8; IQR 28.6 to 41.0) and FLOQSwab (median 35.0; IQR 29.7 to 40.3; P = 0.12).

### 5.1.2 Tolerability

The tolerability of nasopharyngeal sampling was assessed in a subset of 36 participants. On the discomfort scale ranging from 1 (no discomfort) to 5 (extreme discomfort), 22 participants graded Finswab with a lower discomfort value, 10 graded FLOQSwab with a lower discomfort value, and four graded the swabs equally (P = 0.07). When the preference for future nasopharyngeal sampling was enquired, 23 participants preferred Finswab, 12 preferred FLOQSwab, and one participant was inconclusive (P = 0.06). **Figure 12.** shows the assessment of discomfort and swab preference by participants.

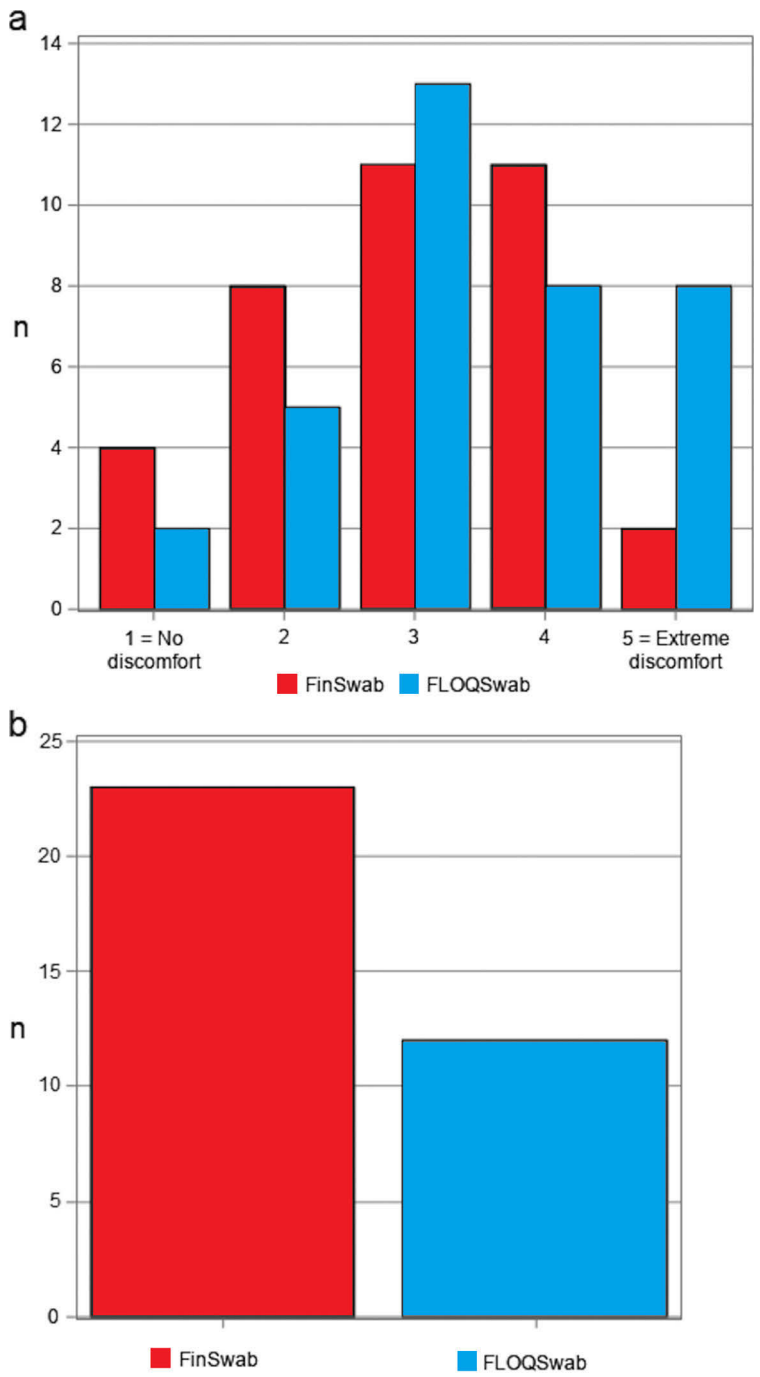


**Figure 12.** (**a**) The discomfort associated with nasopharyngeal sampling by FinSwab and FLOQSwab, assessed by the 36 participants with a discomfort scale from 1 (no discomfort) to 5 (extreme discomfort). (**b**) Preferred nasopharyngeal swab by the participants. Reproduced and modified with the permission of the copyright holders from original study I (CC BY 4.0)

## 5.2 Diagnostic performance and tolerability of saliva

Among the 250 participants recruited to participate in the study evaluating saliva in SARS-CoV-2 diagnostics, 187 (75%) were female, and the mean age was 38 years (range 12 to 77, SD 13). Most individuals (n = 246; 98%) admitted to testing exhibited respiratory symptoms or other signs suggestive of SARS-CoV-2 infection. The median duration of symptoms prior to specimen collection was 2 days (IQR 1 to 3 days). Most saliva specimens were collected in the afternoon, with 177 out of 244 specimens gathered after 12:00 PM (IQR, 11:00 AM to 1:55 PM; range, 9:00 AM to 6:39 PM). The characteristics of the study population and specimen collection details are shown in **Table 4** and the summary of the results is presented in **Table 5.**

**Table 4.** The characteristics of the study population and specimen collection.

| **Characteristic** | **N (%)[A]** |
|---|---|
| Participants | 250 |
| Symptomatic | 246 (98) |
| Asymptomatic | 4 (2) |
| Gender | |
| Female | 187 (75) |
| Male | 63 (25) |
| Age (years), mean (range) | 38 (12 to 77) |
| Saliva collection time, median (IQR) | 12:45 pm (11:00 am to 1:55 pm) |
| Activities[B] that might affect saliva performance 30min prior to collection | |
| Yes | 93 (66) |
| No | 47 (34) |
| Produced saliva volume | |
| ≥ 2ml | 232 (93) |
| 1-2ml | 15 (6) |
| <1ml | 3 (1) |

Abbreviations: IQR, interquartile range

[A]Unless otherwise stated

[B]Includes eating, drinking, smoking, using mouthwash, and brushing teeth

**Table 5.** Main results on diagnostic performance and tolerability of saliva and NPS specimen.

| Outcome | Saliva | NPS | Comparison |
|---|---|---|---|
| SARS-CoV-2 LDT results, No (%) | 250 | 250 | |
| Positive | 134 (54) | 135 (54) | |
| Negative | 116 (46) | 115 (46) | P = 0.76 |
| SARS-CoV-2 LDT E Gene $C_T$ values, Median (IQR) | 26.2 (22.3 to 29.5) | 20.5 (18.5 to 24.4) | P < 0.001 |
| Discomfort evaluation: Preference of sampling method, No (%) | 148 (59) | 62 (25) | P < 0.001 |

Abbreviations: $C_T$, Cycle threshold; IQR, interquartile range; LDT, laboratory-developed test, NPS, nasopharyngeal swab

### 5.2.1 Diagnostic performance

The performance of the saliva specimen compared to the NPS specimen in detection of SARS-CoV-2 by RT-PCR was examined by a laboratory-developed test (LDT) and two commercial tests (PerkinElmer SARS-CoV-2 and SARS-CoV-2 Plus kits). The analyzed target genes included the SARS-CoV-2 E gene, the N/E gene, the ORF1ab gene, the S gene, and the S gene H69-V70 deletion. The outcomes included the number of SARS-CoV-2-positive specimens, SARS-CoV-2 $C_T$ values, and sensitivity and specificity of saliva. The results are summarized in **Table 5**.

Using the LDT, SARS-CoV-2 was detected in 135 participants by NPS and in 134 participants by saliva. With the PerkinElmer SARS-CoV-2 kit, SARS-CoV-2 was detected in 141 participants by NPS and in 136 participants by saliva. With the PerkinElmer SARS-CoV-2 Plus kit, SARS-CoV-2 was detected in 142 participants by NPS and in 136 participants by saliva. The discordant results are shown in the Original Article II (tbl 1). Among the 135 SARS-CoV-2-positive results obtained from NPS using the LDT, 101 (75%) were S gene H69-V70 deletion negative, indicating Omicron BA.2., while 34 (25%) were S gene H69-V70 deletion positive, indicating Omicron BA.1. In comparison to NPS, the sensitivity and specificity of saliva with the LDT were 95.6% (95% CI, 90.6% to 98.4%) and 95.7% (95% CI, 90.2% to 98.6%), respectively.

The $C_T$ values for the LDT E gene were significantly higher in the saliva specimen (median, 26.2; IQR, 22.3 to 29.5) compared to NPS (median, 20.5; IQR, 18.5 to 24.4; $P < 0.001$). The PerkinElmer SARS-CoV-2 Plus kit demonstrated similar results: With N/E gene, $C_T$ values were higher from saliva (median, 26.1; IQR, 22.5 to 30.0) compared to NPS (median, 17.9; IQR, 16.3 to 22.3; $P < 0.001$), and correspondingly with the ORF1ab gene, $C_T$ values were higher from saliva (median, 25.0; IQR, 21.5 to 28.4) compared to NPS (median, 18.5; IQR, 16.7 to 22.0) (**Figure 13).**

The activities conducted 30 minutes prior to saliva collection, including drinking, eating, smoking, using mouthwash, and brushing teeth, were assessed. All 11 discordant results between saliva and NPS of the LDT E gene were from participants who engaged in the listed activities ($P < 0.001$). The $C_T$ values with the LDT E gene were similar, with a mean $C_T$ of 25.6 for participants who engaged in these activities and 26.6 for those who refrained from these actions ($P = 0.280$). The saliva collection time had no impact on the LDT E gene results (OR = 0.522; 95% CI, 0.161 to 1.693; $P = 0.278$) or the $C_T$ values ($r = 0.072$; $P = 0.400$).

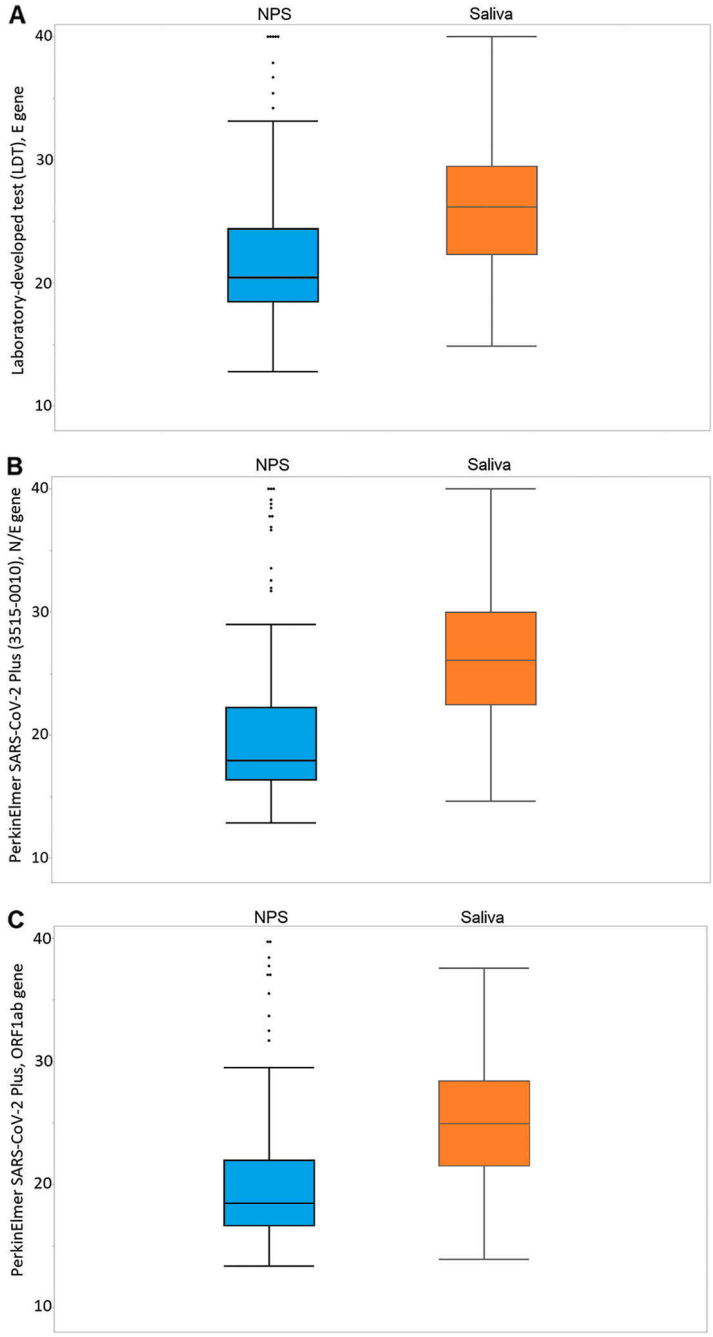


**Figure 13.** The SARS-CoV-2 $C_T$ values from nasopharyngeal swab (NPS) and saliva specimens with (**A**) a laboratory-developed test (LDT), E gene; (**B**) a PerkinElmer SARS-CoV-2 Plus kit, N and E genes; and (**C**) a PerkinElmer SARS-CoV-2 Plus Kit, ORF1ab gene. Medians, interquartile ranges, minimum values, and maximum values are presented. Reproduced and modified with the permission of the copyright holders from the original study II (CC BY 4.0).

### 5.2.2 Tolerability

The tolerability of saliva and NPS collection was assessed via a discomfort scale ranging from 1 (no discomfort) to 5 (extreme discomfort). Among 249 participants who gave discomfort evaluation, 172 participants graded saliva with a lower discomfort value, 17 graded NPS with a lower discomfort value, and 60 graded the methods equally ($P < 0.001$). When the preference for future specimen collection was inquired, 148 participants preferred saliva specimens, 62 preferred NPS, and 39 were inconclusive ($P < 0.001$). **Figure 14.** shows the evaluation of discomfort associated with NPS and saliva by participants.

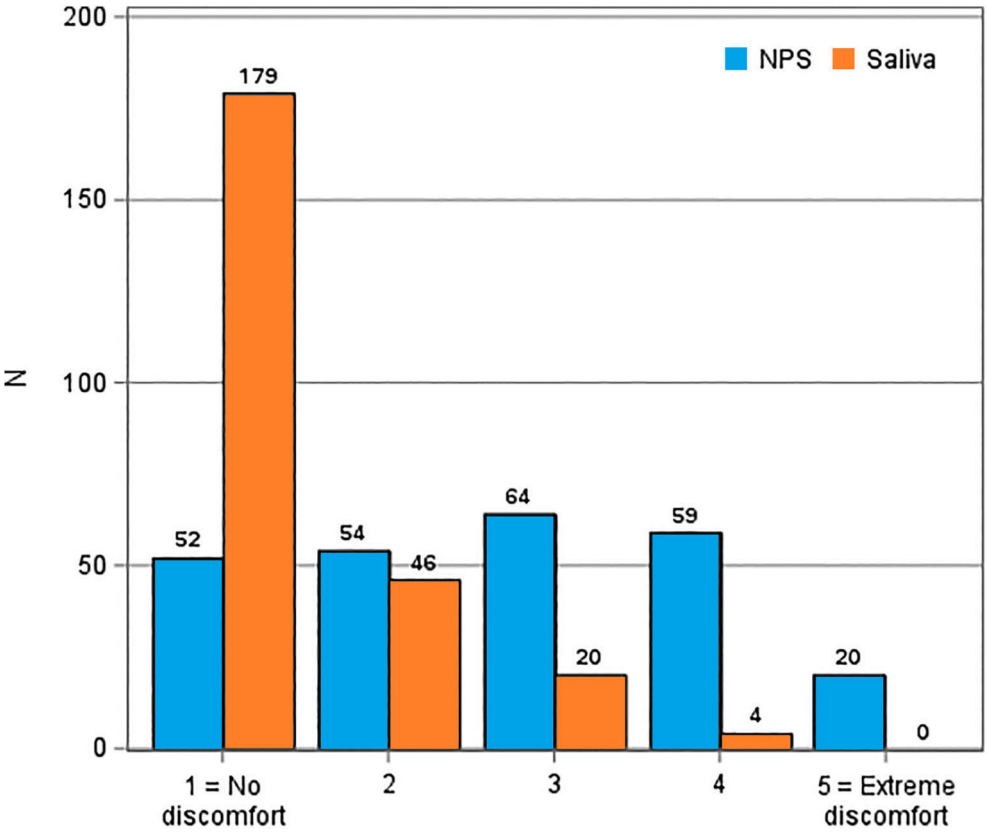


**Figure 14.** The experienced discomfort associated with nasopharyngeal (NPS) and saliva sampling, assessed by participants via a discomfort scale from 1 (no discomfort) to 5 (extreme discomfort). Reproduced and modified with the permission of the copyright holders from the original study II (CC BY 4.0).

## 5.3 Household transmission of SARS-CoV-2-positive and -negative respiratory tract infections

From June to August 2020, 702 individuals were recruited to participate in the study evaluating transmission of respiratory pathogens in households. Two participants were excluded following recruitment. Until April 2022, 76 participants and 20 households dropped out, while 624 participants and 155 households completed the study. Among all 700 participants, 356 (51%) were female and 376 (54%) were children. The age range for all participants was 0.3 to 62 years, the median age for children was 11.1 years (IQR, 9.4 to 12.1 years), and the median age for adults was 43.5 years (IQR, 40.1 to 47.6 years). The minority of the households had more than two children (n = 48, 27%). By the study's end, 274 adults (85%) and 224 (60%) children had received at least two doses of the COVID-19 vaccine. During the study period, 334 individual SARS-CoV-2 infections were observed, of which 300 (90%) were recorded in January-April 2022. The main results are shown in **Table 6**.

**Table 6.** Main results on household transmission of SARS-CoV-2-positive and -negative respiratory tract infections

| Secondary attack rate (95% CI) | SARS-CoV-2-positive | SARS-CoV-2-negative | Comparison[A] |
|---|---|---|---|
| Overall | 41.4 (36.4-46.5) | 24.3 (22.3-26.2) | P < 0.001 |
| Index case | | | |
| Child | 39.6 (33.0-46.3) | 26.9 (24.5-29.3) | P < 0.001 |
| Adult | 43.4 (35.6-51.1) | 18.4 (15.2-21.6) | P < 0.001 |
| Comparison[B] | P = 0.47 | P < 0.001 | |
| Secondary case | | | |
| Child | 42.6 (35.6-49.5) | 25.7 (22.9-28.6) | P < 0.001 |
| Adult | 39.2 (31.9-46.5) | 22.8 (20.1-25.5) | P < 0.001 |
| Comparison[B] | P = 0.51 | P = 0.14 | |

[A]Comparison between SARS-CoV-2-positive and -negative infections
[B]Comparison between children and adults

Individual respiratory infections occurring within 14 days of the initial infection formed infection clusters. After exclusions, 728 respiratory infection clusters in 150 households, comprising 120 (16%) SARS-CoV-2-positive and 608 (84%) SARS-CoV-2-negative clusters, were analyzed. The flow of cluster exclusion is shown in **Figure 15**. SARS-CoV-2-positive clusters comprised 262 individual infections, with

252 (96%) confirmed by SARS-CoV-2-positive specimens, while 10 (4%) were included based on reported symptoms. In SARS-CoV-2-negative clusters, 839 (79%) of 1063 separate infections provided SARS-CoV-2-negative specimens. Of all individual tests incorporated in the clusters (n = 1091), 934 (86%) were SARS-CoV-2 PCR tests, and 157 (14%) were rapid antigen tests.

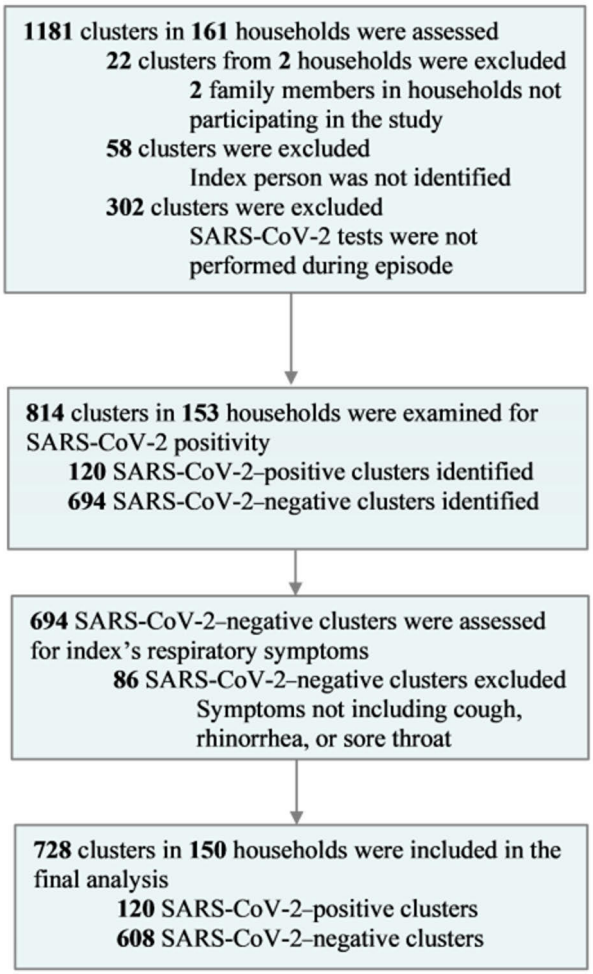


**Figure 15.** The flow of clusters included in the analysis. Reproduced with the permission of the copyright holders from the original study III (CC BY 4.0).

During the study, infections that were negative for SARS-CoV-2 were consistently observed, while a majority (90%) of SARS-CoV-2-positive cases emerged in 2022, coinciding with the rise of Omicron BA.1 and BA.2 variants in Southwest Finland. **Figure 16** illustrates the incidence of SARS-CoV-2-negative

respiratory infections in the study region, and **Table 7** shows the emergence of Omicron variants in the study region.

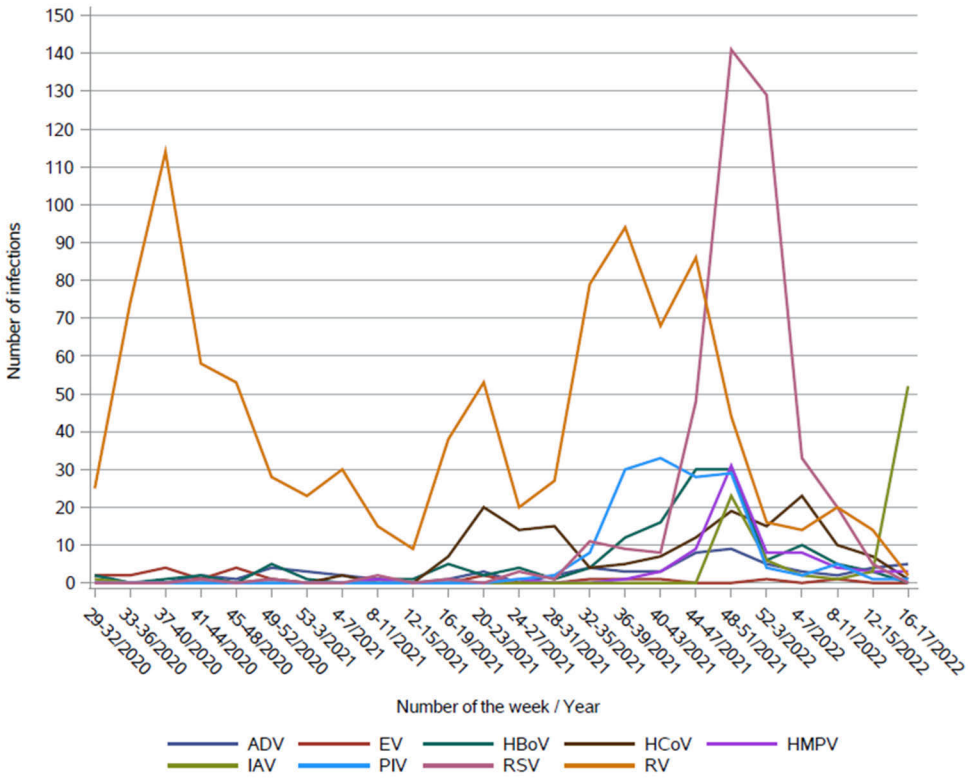


**Figure 16.** Circulation of SARS-CoV-2-negative respiratory viruses in Southwest Finland during the study period. Abbreviations: ADV, adenovirus; EV, enterovirus; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; IAV, influenza A virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus. HCoV includes human coronaviruses OC43, NL63, and 229E. Influenza B viruses (n = 3) are not shown. Data obtained from the Department of Clinical Microbiology, Turku University Hospital. Reproduced with the permission of the copyright holders from the original study III (CC BY 4.0).

**Table 7.** Proportion of Omicron BA.1. and BA.2. variants from week 49/2021 to week 15/2022 in the study region. Data obtained from the Department of Clinical Microbiology, Turku University Hospital. Reproduced with the permission of the copyright holders from the original study III (CC BY 4.0).

| Week/Year | SARS-CoV-2–positive tests, No. | Omicron BA.1, n (%) | Omicron BA.2, n (%) |
|---|---|---|---|
| 49/2021 | 582 | 0 (0) | |
| 50/2021 | 715 | 35 (4.9) | |
| 51/2021 | 978 | 413 (42.2) | |
| 52/2021 | 1831 | 1387 (75.8) | |
| 1/2022 | 2954 | 2626 (88.9) | |
| 2/2022 | 3719 | 3456 (92.9) | |
| 3/2022 | 2911 | 2777 (95.4) | |
| 4/2022 | 1624 | 1532 (94.3) | |
| 5/2022 | 874 | | 39 (4.5) |
| 6/2022 | 2438 | | 185 (7.6) |
| 7/2022 | 2527 | | 248 (9.8) |
| 8/2022 | 2083 | | 372 (17.9) |
| 9/2022 | 2538 | | 1005 (39.6) |
| 10/2022 | 2836 | | 1509 (53.2) |
| 11/2022 | 3032 | | 1975 (65.1) |
| 12/2022 | 2769 | | 2163 (78.1) |
| 13/2022 | 1806 | | 1468 (81.3) |
| 14/2022 | 414 | | 366 (88.4) |
| 15/2022 | 1031 | | 966 (93.7) |

### 5.3.1 Comparisons between SARS-CoV-2-positive and -negative infections

Prevalence and duration of symptoms were compared between SARS-CoV-2-positive and -negative infections. All documented SARS-CoV-2-infections in adults were symptomatic, while 7 (3%) asymptomatic infections in children were excluded from comparisons. The comparison of symptoms between SARS-CoV-2-positive and -negative infections is shown in **Table 8**.

**Table 8.** Prevalence and duration of symptoms in SARS-CoV-2-positive and -negative infections. Reproduced and modified from original study III, with permission from the copyright holders (CC BY 4.0)

| | Fever | Cough | Rhinorrhea | Sore throat |
|---|---|---|---|---|
| Prevalence (n, %) | | | | |
| Total | 329 (25%) | 624 (47%) | 949 (72%) | 888 (67%) |
| SARS-CoV-2-positive | 134 (51%) | 174 (66%) | 179 (68%) | 176 (67%) |
| SARS-CoV-2-negative | 195 (18%) | 450 (42%) | 770 (72%) | 712 (67%) |
| OR (95% CI) | 6.57 (4.63-9.33) | 2.70 (1.98-3.67) | 0.84 (0.62-1.15) | 1.08 (0.79-1.48) |
| P-value (OR)[A] | < 0.001 | < 0.001 | 0.28 | 0.63 |
| Duration (d; median (IQR)) | | | | |
| Total | 2 (2-3) | 5 (3-7) | 5 (4-7) | 3 (3-5) |
| SARS-CoV-2-positive | 3 (2-4) | 4.5 (3-8) | 5 (3-7) | 4 (3-5) |
| SARS-CoV-2-negative | 2 (2-3) | 5 (3-7) | 5 (4-7) | 3 (2-5) |
| P-value (duration)[A] | 0.047 | 0.87 | 0.75 | 0.045 |

Abbreviations: CI, confidence interval; IQR, interquartile range; OR, odds ratio
[A]Symptom prevalences were compared using mixed-effects logistic regression and durations with a linear mixed model.

No difference was observed in the serial interval between SARS-CoV-2-positive and -negative clusters, with the median serial interval being 3 days in both SARS-CoV-2-positive (IQR, 2-4 days) and -negative clusters (IQR, 2-6 days; P = 0.15). The distribution of serial intervals is shown in the Original Article III (fig. 3). Secondary transmission was observed more often in SARS-CoV-2–positive clusters (70.8%; 95% CI, 62.7%-79.0%) compared to SARS-CoV-2–negative clusters (46.4%; 95% CI, 42.4%-50.4%; P < 0.001). The secondary attack rate (SAR) for SARS-CoV-2-positive clusters was 41.4% (95% CI, 36.4%-46.5%) and 24.3% (95% CI, 22.3%-26.2%; P < 0.001) for SARS-CoV-2-negative clusters. SAR was significantly higher in SARS-CoV-2-positive clusters compared to SARS-CoV-2-negative clusters when assessed by any index case or household characteristics (all P < 0.01, **Figure 17**).

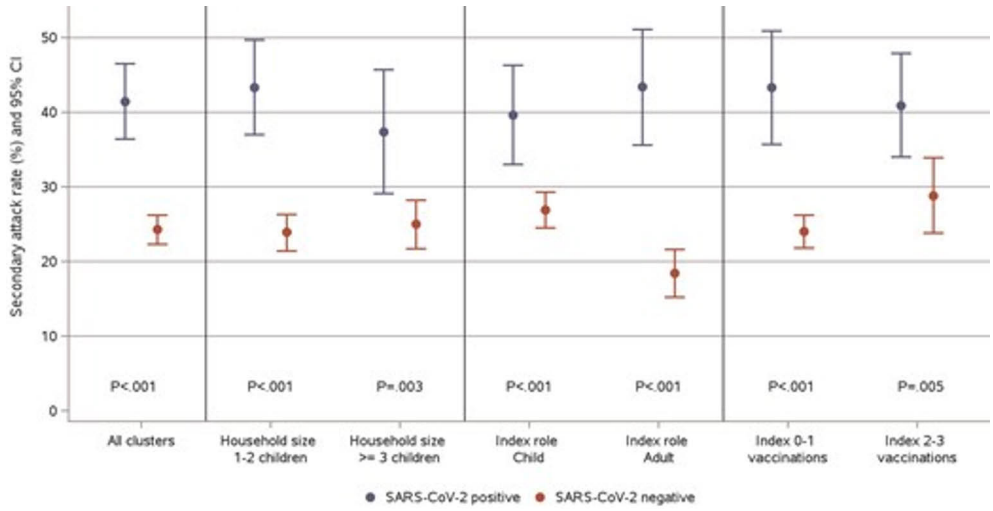


**Figure 17.** Secondary attack rates (SARs) of SARS-CoV-2–positive and SARS-CoV-2–negative infections by household and index case characteristics**.** P values are shown for comparisons between SARS-CoV-2-positive and -negative clusters. In SARS-CoV-2–negative clusters, the SAR was significantly lower when the index case was a child (P < 0.001), while other characteristics showed no significant association when grouped to SARS-CoV-2-positive or -negative clusters. Reproduced and modified with the permission of the copyright holders from the original study III (CC BY 4.0).

### 5.3.2 Comparisons between children and adults

Clinical features of respiratory infections were compared between children and adults separately in SARS-CoV-2-positive and -negative infections. In SARS-CoV-2-positive infections, adults exhibited cough and rhinorrhea more often, while children exhibited fever more often in SARS-CoV-2-negative infections. Duration of any symptom showed no significant difference between children and adults. Comparisons between children and adults are shown in **Table 9**.

**Table 9.** Comparison of symptoms between children and adults. Reproduced and modified from original study III, with permission from the copyright holders (CC BY 4.0)

| Prevalence (n, %) | Fever | Cough | Rhinorrhea | Sore throat |
|---|---|---|---|---|
| SARS-CoV-2-positive | | | | |
| Children | 73 (51%) | 83 (58%) | 83 (58%) | 93 (65%) |
| Adults | 61 (51%) | 91 (76%) | 96 (80%) | 83 (69%) |
| OR (95% CI) | 1.11 (0.62-1.98) | 0.43 (0.23-0.80) | 0.35 (0.18-0.65) | 0.80 (0.43-1.49) |
| P-value (OR)[A] | 0.71 | 0.013 | 0.004 | 0.44 |
| SARS-CoV-2-negative | | | | |
| Children | 135 (21%) | 294 (45%) | 471 (72%) | 445 (68%) |
| Adults | 60 (15%) | 156 (38%) | 299 (73%) | 267 (66%) |
| OR (95% CI) | 1.51 (1.04-2.18) | 1.25 (0.95-1.64) | 0.92 (0.68-1.25) | 1.12 (0.83-1.53) |
| P-value (OR)[A] | 0.029 | 0.11 | 0.61 | 0.46 |

Abbreviations: CI, confidence interval; OR, odds ratio
[A]Symptom prevalences were compared using mixed-effects logistic regression.

SARs between children and adults were compared in SARS-CoV-2-positive and -negative clusters. No significant differences were found when compared within SARS-CoV-2-positive clusters: SAR for the child index case was 39.6% (95% CI, 33.0%-46.3%) while adult index cases resulted in SAR of 43.4% (95% CI, 35.6%-51.1%; P = 0.47). For secondary cases, the SAR for children was 42.6% (95% CI, 35.6%-49.5%), compared to 39.2% (95% CI, 31.9%-46.5%; P = 0.51) for adults. Within SARS-CoV-2–negative clusters, the SAR for child index cases was significantly higher at 26.9% (95% CI, 24.5%-29.3%) compared to 18.4% (95% CI, 15.2%-21.6%; P < 0.001; **Figure 17)** for adult index cases. For secondary cases in SARS-CoV-2-negative clusters, the SAR for children was 25.7% (95% CI, 22.9%-28.6%), compared to 22.8% (95% CI, 20.1%-25.5%; P = 0.14) for adults.

## 5.4 SARS-CoV-2 serology and household transmission

Acute respiratory infections in 175 households with 376 children and 324 adults were prospectively followed from June 2020 to April 2022. SARS-COV-2 seroprevalence was determined three times with an 8-month interval, and five weeks after a SARS-CoV-2 PCR-positive infection in the household. The main results are summarized in **Table 10**.

**Table 10.** Main results on SARS-CoV-2 anti-N-IgG seroprevalence and household transmission using both anti-N-IgG and PCR.

| **Seroprevalence, No. (%)** | |
|---|---|
| June-December 2020 | 3 (0.5) |
| February-May 2021 | 6 (1.0) |
| November 2021- February 2022 | 8 (1.9) |
| Secondary attack rate, % (95% CI) | |
| Overall | 59 (51-68) |
| Index case | |
| Child | 48 (37-59) |
| Adult | 77 (61-89) |
| Unvaccinated | 53 (40-67) |
| Vaccinated | 63 (50-75) |
| Secondary case | |
| Child | 66 (52-77) |
| Adult | 49 (36-62) |
| Unvaccinated | 83 (61-95) |
| Vaccinated | 52 (41-62) |

### 5.4.1 Seroprevalence

The seroprevalence of participants was measured at three distinct time points: during recruitment from June to December 2020, from February to May 2021, and from November 2021 to February 2022. The seroprevalence of SARS-CoV-2 anti-N-IgG was 0.5% (3 of 661) at recruitment, 1.0% (6 of 609) during the second sample collection, and 1.9% (8 of 418) in the third collection period. For SARS-CoV-2 anti-S-IgG, the prevalences were 2.1%, 6.4%, and 80.0%, respectively. The rise in anti-S-IgG seroprevalence indicated the progression of COVID-19 vaccinations among the population.

### 5.4.2 Seroconversion in SARS-CoV-2 PCR-positive households

Seroconversion was assessed in 55 households and 207 individuals following the detection of SARS-CoV-2 infection via PCR in at least one household member. None of these participants exhibited prior seropositivity by anti-N-IgG in seroprevalence specimens. The median duration from the positive SARS-CoV-2 PCR test of the index case to serum collection was 39 days (IQR 36-42 days). A total of 109 (53%) participants were then anti-N-IgG positive, i.e., were seroconverted. Children were more often seropositive (n = 65, 59%) than adults (n = 44, 45%) and

serum collection after 1st of February 2022 was associated with higher seroconversion rate (n = 93, 57%) than before 1st of February 2022 (n = 16, 37%). Anti-N-IgG levels in children and symptomatic participants were higher compared to adults and asymptomatic participants (**Figure 18)**.

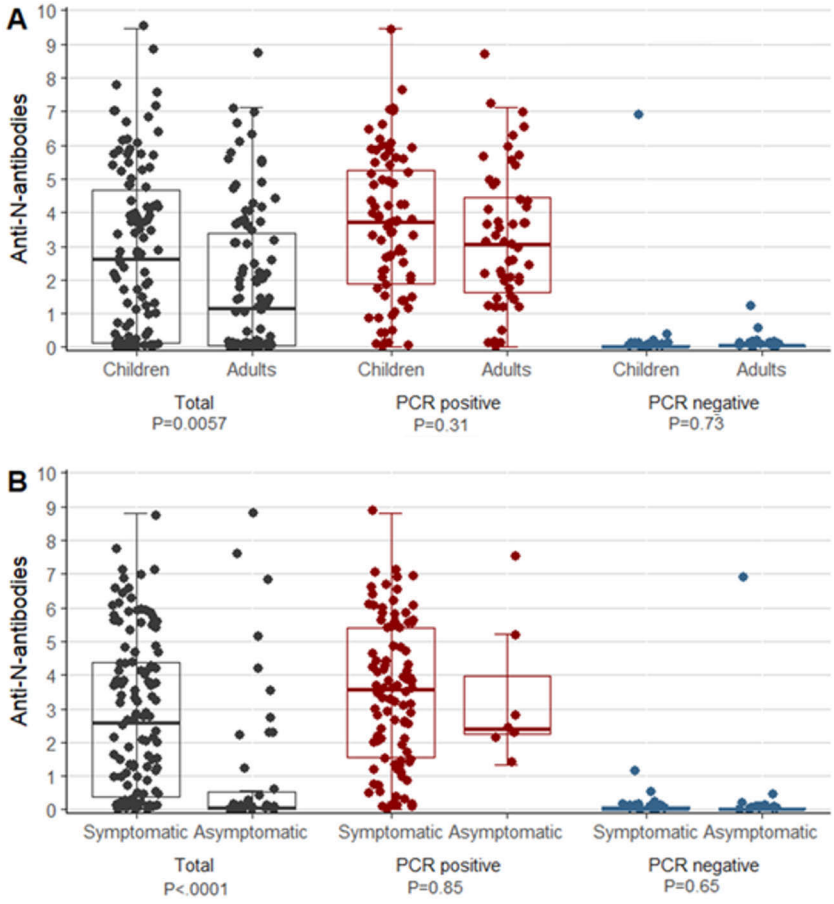


**Figure 18**. Anti-N-IgG values of 207 participants after a PCR-positive SARS-CoV-2 infection in the household. **A**. Shows comparisons between children and adults and **B** shows comparison between symptomatic and asymptomatic participants. No significant differences were observed between children and adults and between asymptomatic and symptomatic participants when compared in groups of PCR-positive and PCR-negative participants. Anti-N-IgG values were significantly higher in children than in adults (P<0.01) and higher in symptomatic participants compared to asymptomatic (P <0.01), when compared among all participants.

A total of 185 participants were examined using both PCR and anti-N-IgG. Anti-N-IgG was positive for 104 (56%) and PCR for 127 (68%) participants. Of these, 24 of

25 discrepant results were PCR-positive/anti-N-IgG-negative, and one was PCR-negative/anti-N-IgG-positive. The sensitivity for anti-N-IgG was 0.81 (95% CI 0.74-0.88), and the specificity was 0.98 (95% CI 0.95-1.00) when using PCR as a reference. A total of 60 participants were examined using both home-performed SARS-CoV-2 antigen test and anti-N-IgG. Anti-N-IgG was positive for 39 (65%) and the antigen test for 37 (62%) participants. Of these, 9 of 20 discrepant results were antigen-positive/anti-N-IgG negative, and 11 were antigen-negative/anti-N-IgG-positive.

### 5.4.3 Household transmission

SARs were assessed in 55 households with at least one PCR-positive SARS-CoV-2 case. SARs were similar using the combination of PCR and anti-N-IgG results or relying solely on PCR (Original Article IV, fig 2.). Using both the PCR and anti-N-IgG, the overall household SAR was 59% (95% CI, 51%-68%; **Figure 19**). The SAR for adult index cases was higher at 77% (95% CI, 61-89%) in comparison to 48% (95% CI, 37-59%) for child index cases (P<0.01). The SAR for adult secondary case was 49% (95% CI 36%-62%) and for the child secondary case 66% (95% CI 52-77%; P = 0.07).

Using both the PCR and anti-N-IgG, the SAR for unvaccinated index cases was 53% (95% CI, 40-67%) and for vaccinated index cases 63% (95% CI, 50-75%). The SAR for vaccinated secondary cases was lower at 52% (95% CI, 41-62%) in comparison to 83% (95% CI 61-95%) for unvaccinated secondary cases (P<0.01).

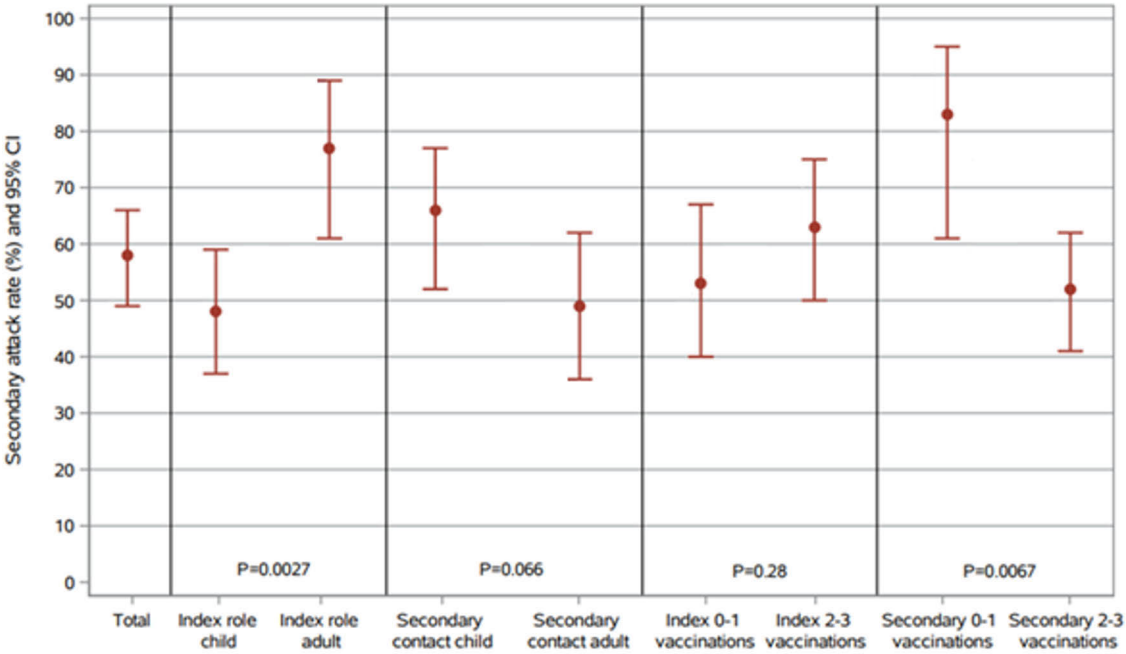


**Figure 19.** Secondary attack rates (SARs) of SARS-CoV-2 PCR and/or N-IgG positive cases assessed by index and secondary case characteristics. P values show the comparison of SARs between characteristics. SAR was significantly higher when the index case was an adult and the secondary case was insufficiently vaccinated (P<0.01). Other differences between characteristics were not statistically significant.

### 5.4.4 Sensitivity analysis

Following the initial analyses using the manufacturer's cutoff value, sensitivity analysis was conducted using a lower anti-N-IgG cutoff value. The cutoff value was set at +2 SD from the mean anti-N-IgG levels at recruitment, before COVID-19 vaccinations and extensive circulation of SARS-CoV-2.

Using the lower cutoff value, anti-N-IgG seroprevalences at recruitment, at second sample collection, and at third sample collection were 0.9%, 1.7%, and 2.9%, respectively. In comparison of PCR and anti-N-IgG results with 185 participants, the lower cutoff value resulted in 116 (63%) anti-N-IgG-positive cases, while 127 (69%) were PCR positive. Of 15 discrepant results, 13 were PCR-positive/anti-N-IgG-negative and 2 were PCR-negative/anti-N-IgG-positive. The sensitivity for anti-N-IgG, using a lower cutoff value, was 0.90 (95% CI 0.83-0.94), while the specificity was 0.97 (95% CI 0.88-0.99) when using PCR as a reference. In analysis of SAR, lower cutoff value led to three more SARS-CoV-2-positive cases, leading to minor increases in SAR: Overall SAR was 61% (95% CI, 52-69%), adult index case SAR was 78% (95% CI, 62-89%), and child index case SAR was 50% (95% CI 39-61%).

# 6 Discussion

## 6.1 Molded plastic nasopharyngeal swab in respiratory virus detection

Two distinct techniques can be utilized to produce NPS: 3D printing and plastic injection molding. The prototypes of the study swab were produced by 3D printing. The 3D printing technology enabled manufacturing computer-generated prototypes of NPS for preclinical evaluations without delays. Additionally, prior studies conducted on 3D-printed NPS had demonstrated that 3D-printed NPS could equal the standard NPS in the detection of SARS-CoV-2 (Alghounaim et al., 2020; Arjunan, 2021; Callahan et al., 2020; Decker et al., 2020; Oland et al., 2021). However, although 3D printing can swiftly be employed for the production of NPS, the capacity of 3D printers for large-scale mass production of NPS is ultimately limited (Ngo et al., 2018; Tack et al., 2016). Thus, the final product was chosen to be manufactured by plastic injection molding. This method necessitates a greater initial investment but is more conducive to large-scale mass production compared to 3D printing. Moreover, NPS manufactured using the plastic injection molding technique should also demonstrate efficacy based on their material properties. Injection molding facilitates the use of the same types of plastics as those employed in 3D printing, while also providing access to a wider range of plastics. (Zema et al., 2012) To our knowledge, no studies on NPS manufactured by plastic injection molding have been conducted prior to our study.

During the execution of the study, the low prevalence of SARS-CoV-2 in Finland resulted in the acquisition of only four SARS-CoV-2-positive specimens by both swabs, with comparable $C_T$ values observed. However, the evaluation of another single-stranded positive-sense RNA virus, rhinovirus (n=30), demonstrated that the detection rates and $C_T$ values were similar in FinSwab and reference swabs. These findings indicate that the diagnostic performance for SARS-CoV-2 is presumably satisfactory as well, although the study was underpowered to specifically assess that. Furthermore, the $C_T$ values of human β-actin mRNA were comparable between the swabs, supporting the effective collection of cellular material through sampling. However, while human β-actin mRNA is commonly used gene for internal controls,

the method lacks thorough research, and some studies indicate that it may be unreliable (Selvey et al., 2001).

Comparative studies of the tolerability of NPS specimen collection are lacking. In this thesis, similar results were obtained for FinSwab and FLOQSwab. Although 23 (64%) of 36 participants preferred FinSwab, the difference was not significant. In an Australian study by Williams et al., sample collection by 3D-printed swabs was less uncomfortable for 67% of the participants, corresponding to our results (Williams, Bond, Isles, et al., 2020).

## 6.2 Saliva in SARS-CoV-2 diagnostics

Several recent studies have shown that the diagnostic efficacy of saliva specimen is satisfactory for SARS-CoV-2 diagnostics. However, in line with the findings of the present thesis, most studies have shown lower $C_T$ values for NPS compared to saliva (Callahan et al., 2021; Migueres et al., 2020; Poukka et al., 2021; Salmona et al., 2022; Uršič et al., 2022; Williams, Bond, Zhang, et al., 2020). Contrastingly, some studies suggest that saliva might be a more sensitive matrix to detect the Omicron variants of SARS-CoV-2 (Cornette et al., 2022; Lai et al., 2022; Marais et al., 2021), while other studies continue to reveal lower $C_T$ values for NPS despite Omicron emergence (Salmona et al., 2022; Uršič et al., 2022). Our findings correspond with the latter as lower $C_T$ values were observed with NPS despite the Omicron BA.2. variant being dominant in Finland during the study's conduction.

This thesis evaluated the factors that may influence the sensitivity of saliva specimens. We revealed that the timing of the specimen collection, as well as eating, drinking, smoking, using mouthwash, or brushing teeth 30 minutes prior to the saliva collection, had no effect on the $C_T$ values obtained via saliva specimens. These findings are consistent with the research conducted by Callahan et al., which involved 385 paired saliva and NPS specimens (Callahan et al., 2021). In contrast, some studies have reported higher viral loads in saliva specimens collected early in the morning compared to those collected later in the day (Bastos et al., 2021; Hung et al., 2020). Notably, our study identified an intriguing finding: All 11 discordant results between saliva and NPS of the LDT E gene were from participants who had engaged in activities that might compromise saliva sensitivity 30 minutes prior to saliva collection. Furthermore, this same effect was observed with both commercial testing platforms. Even though $C_T$ values were similar, the discordant results suggest that the specified activities should remain prohibited before saliva sampling.

Although saliva collection has been considered easy and well-tolerated, the study by Uršič et al. reported that some participants experience difficulties in providing a sufficient amount of saliva for diagnostic purposes (Uršič et al., 2022). Correspondingly, 18 participants (7%) in our study were unable to provide the

required 2 ml of saliva. Additionally, NPS collection was preferred by 62 (25%) participants, which is a notable number considering that only 17 (7%) participants reported higher discomfort values for saliva. Our findings suggest, that while saliva is the more preferred specimen collection method overall, a significant minority still opts for NPS, likely due to its expedited collection process.

## 6.3 The role of children in the transmission of SARS-CoV-2-negative and -positive infections

In this thesis, the rates of SARS-CoV-2-positive and -negative infections, along with the clinical features, were prospectively evaluated with 700 participants consisting of school-aged children and their family members. Despite the long follow-up period from June 2020 to April 2022, most (90%) SARS-CoV-2 infections occurred during the Omicron BA.1. and BA.2. dominance at the beginning of the year 2022. Contrastingly, SARS-CoV-2-negative infections occurred consistently during the study period, with rhinovirus potentially responsible for a major number of infections based on the data on circulating viruses.

The household transmission of SARS-CoV-2-positive infections was high in our study. In study III, we observed the overall SAR of 42% despite the extensive vaccination rate in our population, which aligns with the previous reports on transmissibility of Omicron variants. In study III of this thesis, no statistically significant differences were identified in SAR between children and adults, between vaccinated and unvaccinated, or between households of varying sizes. These findings are consistent with prior studies reporting limited vaccine effectiveness against the transmission of the Omicron variant and the absence of differences in transmissibility between children and adults with SARS-CoV-2 Omicron infection (F. Chen et al., 2022; Fiolet et al., 2022; Madewell et al., 2022; Menni et al., 2022).

This thesis also addressed the transmission of SARS-CoV-2-negative infections in households. Throughout the follow-up period, a variety of respiratory viruses circulated. Evidence derived from data on circulating viruses in Finland indicates that rhinoviruses may have been the most prevalent SARS-CoV-2-negative pathogens in our findings, followed by RSV, parainfluenza, and seasonal coronaviruses. As virus transmission has been traditionally measured in community settings with R0 values rather than with SAR within household environments, the existing literature on SARs for respiratory viruses aside from SARS-CoV-2 is relatively limited, particularly regarding the studies that investigate the role of children in the transmission process. Concerning our results on SARS-CoV-2-negative infections, we observed a significantly higher SAR among child index cases in comparison to adult index cases. Correspondingly, Peltola et al. demonstrated that transmission rates for rhinovirus-positive child index cases were elevated among

siblings in contrast to parents (Peltola et al., 2008). Regarding seasonal influenza, Nukiwa-Souma et al. found that SAR for influenza-like illness was highest among young children aged 1-4 years. However, the characteristics of the index cases demonstrated no significant effect on SAR (Nukiwa-Souma et al., 2012). In the context of the 2009 H1N1 pandemic influenza, Sugimoto et al. found that children were more susceptible to secondary attacks than adults (Sugimoto et al., 2011). In our study, we found that children and adults exhibited equal susceptibility to respiratory infections within SARS-CoV-2-negative households.

## 6.4 Serological results and SARS-CoV-2 household transmission

The seroprevalence of SARS-CoV-2 remained notably low from June 2020 until the beginning of 2022 in our study. During the winter-spring period of 2022, a substantial increase in PCR-confirmed SARS-CoV-2 infections within the participant households was observed, aligning with the rapid dissemination of the Omicron variants BA.1 and BA.2 in Finland at the time (Ahava et al., 2022; Solastie et al., 2023). Consequently, 79% (163 of 207) of seroconversion samples in our study were collected after February 1st, 2022. Higher seroconversion rates were observed in these households compared to samples collected prior to this date. This was despite the high vaccination-induced immunity, as demonstrated by 80% of participants being anti-S-IgG positive at the last seroprevalence samples. This finding is consistent with existing literature that highlights the increased transmissibility and decreased vaccine efficacy associated with the Omicron variant (Baker et al., 2022; F. Chen et al., 2022; Madewell et al., 2022; Manathunga et al., 2023).

Most SARS-CoV-2 PCR-positive participants exhibited seroconversion for anti-N-IgG, regardless of their vaccinations, age, or the infection symptoms. This finding indicates the presence of strong immune responses to natural infection, a conclusion that aligns with observations from other studies, at least within a short time frame following acute infection (Di Chiara et al., 2022; Gudbjartsson et al., 2020; Toh et al., 2022). Studies have reported distinct antibody profiles between adults and children, with younger children exhibiting the highest antibody levels (Di Chiara et al., 2022; Yang et al., 2021). In our study, however, anti-N-IgG levels were similar between children and adults. This discrepancy may be attributed to the age distribution in our study population, as the study's children were mostly over 10 years old, and the proportion of preschool-aged children was low.

In this study, we observed only minor increases in household transmission of SARS-CoV-2 when serologically positive cases were added to PCR detections. Sensitivity of the SARS-CoV-2 PCR has been estimated to be around 88 to 95%, but

repeated PCR tests at one week in our study may have increased the sensitivity of the PCR, thus limiting the appearance of PCR-negative/serology-positive cases (Binny et al., 2022). Additionally, the cutoff value of the commercial anti-N-IgG test may have been too high to detect low positives, as lowering the cutoff value in our sensitivity analysis provided 12 more anti-N-IgG-positive results, of which only one was PCR-negative. Nevertheless, results remained essentially similar by both cutoffs. SAR values were high in the population assessed for seroconversion. In Study IV of this thesis, adult index cases demonstrated higher infectivity than children, while in Study III of this thesis no difference between children and adults was observed. However, both studies showed that, unlike other respiratory viruses, children are not the major source of secondary SARS-CoV-2 infections in the family. These partly discrepant results, even within the same cohort, illustrate the variability of SAR across different studies, despite both SARs in our research being consistent with existing literature concerning Omicron transmission (Baker et al., 2022; F. Chen et al., 2022; Madewell et al., 2022). Previous literature has indicated that adults were more infectious with earlier SARS-CoV-2 variants; however, the disparity in infectivity between children and adults appears to have diminished with the emergence of the Omicron variant (F. Chen et al., 2022; Goldstein et al., 2021; Miller et al., 2021).

## 6.5 Strengths and limitations of the studies

This thesis and the studies it consists of have several strengths. All the studies were conducted in real-world settings, the participants were prospectively recruited, and the number of attending participants was desirable. In studies III and IV, participants were directly contacted by the study nurse concerning specimen and data collection throughout the follow-up period. Furthermore, participants retained continual access to the study nurse and had the opportunity to initiate direct communication as needed. This approach may have contributed to the high follow-up rate despite the follow-up lasting nearly two years.

In Study I, several respiratory viruses and human β-actin mRNA were analyzed and compared. In study II, all paired samples were analyzed by three distinct tests, and for several target genes of SARS-CoV-2. In studies III and IV, the data were derived from electronic medical records in addition to weekly questionnaires. The inclusion of several outcomes and data sources adds to the reliability and versatility of the studies. Additionally, samples aside from home antigen tests in Study III were collected and supervised by well-trained professionals. Tolerability analyses in Studies I and II provide a new and often overlooked perspective on the discomfort of specimen collection, and the randomization of collection in Study I enhances the

reliability of the evaluation. To our knowledge, the Study III is the first to compare the transmission of SARS-CoV-2-positive and -negative infections in households.

This thesis has several limitations. In Studies I and II, the assessment of discomfort may have been affected by selection bias. In Study I, people who find NPS specimen collection unpleasant could have been more unlikely to participate in the study with two NPS collections, while in Study II, they might have been more eager to participate in a study that evaluates alternative collection methods.

We also note a few limitations associated with potential measurement bias. Due to the comparably low epidemic activity of SARS-CoV-2 in Finland during the Study I, only four SARS-CoV-2-positive specimen pairs were obtained from 112 participants. However, rhinoviruses were found frequently enough for comparisons. Using of human β-actin mRNA may also be susceptible to measurement bias, as this method has not been thoroughly studied. In Study II, a few saliva specimens did not meet the minimum requirement of 2 ml but were nevertheless included in the analysis. This might have decreased the sensitivity and $C_T$ values obtained from saliva. Furthermore, the nucleic acid extraction and RT-PCR were not performed on the specimen collection day. However, this delay was consistent for both NPS and saliva specimens. In study IV, two commercial serological assays were used, but only the anti-N-IgG assay can detect an immune response to natural infection in the vaccinated population. Additionally, the cutoff value of anti-N-IgG set by the manufacturer may have been too high to detect low positives, resulting in underestimation of serologically positive cases. However, we performed a sensitivity analysis with a lower cutoff value for anti-N-IgG. The cutoff value was set at +2SD of anti-N-IgG levels at recruitment, when the study population was mostly SARS-CoV-2 naïve and unvaccinated. We acquired 12 new anti-N-IgG-positive cases of which 11 were PCR-positive, further supporting that manufacturer's cutoff value may have been too high. Regardless of the cutoff value used, the results remained essentially similar.

Regarding studies III and IV, recall bias is possible as daily symptoms were monitored weekly. Additionally, 90% of SARS-CoV-2 infections in households occurred in 2022, limiting our results in studies III and IV mainly to the Omicron variants of SARS-CoV-2. Nevertheless, given the low number of SARS-CoV-2 infections before the emergence of the Omicron variant, the study population represents a particularly interesting cohort with a low natural immunity coupled with high vaccine-induced immunity.

The potential for misclassification of clusters in Study III is a limitation. In the study, infections negative for SARS-CoV-2 were not specifically identified for other pathogens, and all secondary cases were not assessed using the SARS-CoV-2 PCR. Furthermore, the incorporation of home antigen tests may have contributed to the misclassification of certain infections or clusters. Nevertheless, it should be

highlighted that the majority of clusters underwent testing with the SARS-CoV-2 PCR, and home antigen tests represented a minority of the specimens. Although SARS-CoV-2 PCR demonstrates high diagnostic sensitivity, the real-life clinical sensitivity of the method may be lower, and some false negative cases might have been misclassified. In a theoretical context, misclassifying infections would be expected to diminish the observed differences between the groups. Despite the possibility of some infections being misclassified, significant differences were observed between SARS-CoV-2-positive and -negative infections, further supporting the validity of the classification.

The SAR varies among different study populations, settings, and viral variants. Thus, the generalizability of studies conducted within a single time period and geographical location is limited.

## 6.6 Future aspects

After the pandemic, SARS-CoV-2 has now become a persistent, circulating virus with never-ending evolution. It is crucial to stay vigilant about SARS-CoV-2 developments, as it still causes localized epidemics and represents a significant burden on healthcare systems.

Despite the official conclusion of the SARS-CoV-2 public health emergency on May 5, 2023, the volume of nasopharyngeal swabs collected has not returned to pre-pandemic levels (Lenharo, 2023). Numerous countries, especially high-income economies such as Finland, have sustained their policy of conducting NPS testing for all hospitalized individuals at risk of developing severe respiratory infections. In Finland, testing frequently includes RSV, influenza A and B viruses, and SARS-CoV-2. Additionally, the use of respiratory panel tests that assess a wider range of respiratory pathogens has increased compared to the levels seen before the pandemic. Considering this and the inevitability of future pandemics, more studies on plastic injection molded NPS and saliva are required. Regarding NPS, studies that assess a wider range of respiratory pathogens are necessary to validate the performance of such NPS further. As the production of NPS is still limited to a few factories worldwide, improving our knowledge while maintaining preparedness for initiating NPS manufacturing is essential to ensure the national security of supply chains. Regarding saliva, the performance among distinct pathogens can differ notably, highlighting the need for more comprehensive studies on a broader spectrum of pathogens. Considering that some individuals are unsuitable for NPS collection due to predisposing medical conditions, it is vital to constantly update our knowledge on saliva specimen's performance as new pathogens emerge. In the near future, many individuals may be able to select the sample collection method

depending on their preference, while professionals can focus on maintaining specimen efficacy.

Despite the numerous studies conducted on the transmission of SARS-CoV-2, the precise mechanisms of spread across various scenarios remain inadequately understood. Considering many other respiratory viruses, the understanding of these transmission mechanisms appears to be even less comprehensive. Moreover, as the results of these studies vary significantly based on the study design, they are hardly comparable. Thus, long-term prospective studies of transmission in households with the identification of several respiratory pathogens are warranted. However, conducting such research with a decent number of participants, long follow-up periods, and multiple specimen collections demands considerable resources and is burdensome for attendees. International collaboration could increase the power to detect variations among different transmission scenarios and pathogens. On the other hand, developing technology may aid us in conducting studies while reducing attendees' burden. Potential technological innovations include easier specimen collection methods and easier symptom monitoring. For example, currently, smart rings can measure heart rate, detect dysrhythmia, monitor sleep, and measure temperature (Fiore et al., 2024). With further advancements, smart rings may be able to detect subtle human signals that indicate the onset of infections. In the context of medical research, those signals could be used to target specimen collection.

The rising frequency of pandemics observed over the past few decades indicates that the threat of a new pandemic is imminent. Pandemics are closely interconnected with other issues that pose a threat to human existence. Factors such as overpopulation and urbanization increase the transmission of pathogens among humans. The expansion of international travel aids the swift spread of emerging pathogens across the globe. Deforestation forces wildlife closer to human populations, creating more opportunities for zoonotic spillovers to humans. Climate change expands the geographical area where tropical and subtropical infections can flourish, and the following extreme weather events can create conditions where pathogens are spread intensively (Carlson et al., 2022). The accelerating loss of biodiversity disrupts the flow of viruses between the hosts and vectors, the consequences of which are inadequately understood. (Haddad et al., 2015)

However, individual actions can reduce the risk of pandemics while simultaneously mitigating other factors that impact the well-being of both humanity and nature. By reducing overconsumption, particularly the consumption of meat, we can reduce the impacts of livestock production on climate change and deforestation while also mitigating the likelihood of emerging pandemics linked to farmed animals. By traveling less, we can minimize the risk of spreading pandemic pathogens and simultaneously reduce our carbon footprint. By protecting existing forests and establishing new conservation areas, we can reduce the occurrence of

abnormal interactions between wild animals and humans. Finally, by focusing on our efforts to develop and favour green technology, we ultimately contribute to the welfare of ourselves, nature, and the globe.

# 7 Conclusions

In this thesis, we compared molded plastic nasopharyngeal swab (Finswab) to reference NPS in 112 participants, and saliva to NPS in 250 participants at the Turku University Hospital COVID-19 testing station. We found that the plastic injection molding technique is a viable alternative for the mass production of NPS utilized in respiratory virus diagnostics and saliva specimen can serve as an alternative to an NPS specimen for the diagnosis of SARS-CoV-2. We observed that both the molded plastic nasopharyngeal swab and saliva were equivalent to that of the conventional brush-like swab in diagnostic efficacy. However, we found significantly lower $C_T$ values for NPS compared to saliva, indicating that NPS may possess a slightly higher sensitivity than saliva for SARS-CoV-2 diagnostics. In terms of tolerability, NPS was equivalent and saliva superior when compared to reference swab. Additionally, we showed that both the conventional and the study swabs can be effectively transported in dry transport tubes and VTM without significantly compromising diagnostic performance.

In this thesis, we prospectively followed acute respiratory tract infections of 700 participants in 175 households with school-aged children from June 2020 to April 2022. SARS-CoV-2-PCR was obtained from symptomatic and exposed participants. SARS-CoV-2 seroconversion was evaluated five weeks after the SARS-CoV-2-positive infection in the household. We discovered that the household transmission of SARS-CoV-2 was higher when compared to the transmission of SARS-CoV-2-negative infections. Transmission of and susceptibility to SARS-CoV-2 Omicron variants were similar between children and adults, and between vaccinated and unvaccinated participants. In the smaller subpopulation (n= 207) not assessed for symptoms and studied with both PCR and serology, adults were more effective transmitters of SARS-CoV-2 than children, and unvaccinated participants showed greater susceptibility to SARS-CoV-2. In this subpopulation, the inclusion of serological testing resulted only in minor increases in identifying secondary attacks. Regarding SARS-CoV-2-negative infections, we discovered that children contributed more often to transmitting infections to other household members. Susceptibility to SARS-CoV-2-negative infections was equal for children and adults.

In this thesis addressing novel specimen collection methods and the transmission of SARS-CoV-2 and other respiratory pathogens, we showed that Finswab and saliva can both be used in SARS-CoV-2 diagnostics. We discovered that the household transmission of SARS-CoV-2 Omicron variants was higher compared to other respiratory infections, and children were more infectious than adults for SARS-CoV-2-negative infections. Serology resulted in only a minor increase in identifying secondary attacks within households where participants underwent PCR testing twice.

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*Jaakko Ahti*

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