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## Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in Austria: Evidence of effective sentinel surveillance screening in primary care

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37	49	ABSTRACT
38 39	50	<b>Objectives:</b> We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
40 41	51	regional COVID-19 outbreak in Austria.
42	52	Design: Prospective cohort study.
43 44	53	Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.
45 46	54	Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
47 48	55	April, 2020.
40 49	56	Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
50 51	57	chain reaction (RT-qPCR).
52	58	Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the
53 54	59	outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
55	60	with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
56	61	convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
57 58	62	accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.
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63 Results: Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent), 64 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all 65 acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of 66 symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days 67 (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
 surveillance of coronavirus.

74 Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments for nasopharyngeal swabs, and people testing RT-PCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusions on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

#### 85 INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 25 million cases, and over 850,000 deaths reported as of August 31, 2020. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.<sup>1</sup> In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,<sup>2</sup> infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that,<sup>3,4</sup> and gradually declining over 7-10 days.<sup>5,6</sup>

96 SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a
97 large number of people. Although most infections remain isolated cases, a small number of individuals (10%)
98 may cause up to 80% of secondary transmissions.<sup>7</sup> Undocumented infection may constitute the majority of cases
99 (86%), causing more than half (55%) of all documented infections.<sup>8</sup> Superspreading events have been reported
100 from across the globe, and countries achieving early viral suppression took rapid and decisive action to implement

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101 comprehensive case identification and testing, combined with contact tracing and isolation.<sup>9,10</sup> For epidemic 102 control of COVID-19, the effective reproduction number,  $R_e$ , needs to be less than 1; the presence of undetected 103 and persistent infection within the population, even if very small, can increase  $R_e$  and induce a secondary peak of 104 infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward 105 transmission and controlling the virus to protect the public.<sup>11</sup>

11 106

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.<sup>12</sup> Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.<sup>12,13</sup> Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and via mobile units.<sup>14</sup> The first death from COVID-19 associated complications occurred on March 12, 2020, and as of August 31, 27,166 cases and 733 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.<sup>15-17</sup> The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."<sup>18</sup> However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.<sup>19</sup> In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.20 The new service supplemented the existing national health hotline for people at risk of COVID-19.<sup>21</sup> RT-qPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.22 Early detection of SARS-CoV-2 is essential for effective contact tracing,<sup>23</sup> and whole genome sequencing may provide data on dynamics of transmission.13 

43 127

The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak. 

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139	METHODS

# 5 140 **Setting** 6

This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria, people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and home self-isolate and self-care.

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#### 151 Design

We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2 RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and seropositivity data were collected to compare two groups within this cohort of patients:

- Patients testing RT-qPCR reactive at presentation with acute disease
- Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).

We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR
and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6
weeks after the acute illness, irrespective of the RT-qPCR result.

#### 161 Intervention

162 On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza 163 Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.

Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2 infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number of days from the first patient to the last patient testing RT-qPCR reactive at the GP. 

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173 Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening
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174 for patients attending sentinel GPs and paediatric practices. Between November and March of each year,

participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms.
Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue
cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal
influenza virus activity in the country.

# 12 180 Outcome measures

We characterise the outbreak using the following four testing, clinical and viral genomic outcomes: A) The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative; B) The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters); C) The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent seropositivity (confirmed infection) to determine any potential correlation between these stages of disease; and D) the viral clades detected in the outbreak.

#### 191 Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

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42	199	Testing

#### 44 200 RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).<sup>22</sup> RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection. 

53 206 Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),<sup>24</sup> and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.<sup>25</sup> Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of 

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SARS-CoV-2. Reagent wells of the EDI<sup>™</sup> Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of CoV2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA, IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI). 

21 224 Neutralising antibody assay

Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers  $\geq 10$  were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.<sup>26</sup> 

#### 233 Statistical analysis

234 We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following
 235 four outcomes:

Outcome A: We tested the diagnostic accuracy of RT-qPCR, by determining its sensitivity and specificity. To do
this, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody
positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive,
antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive).

**Outcome B:** We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) vs. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent). 

59 248 Outcome C: Multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR
 60 249 reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and

250 the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status 251 respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody 252 positive) and all patients with confirmed infection by calculating the correlation coefficient r, and estimating the 253 95% CI.

**Outcome D**: For clade analysis, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.<sup>13</sup> The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).<sup>27</sup> Sequences were aligned in MEGA7 and non-synonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.<sup>28</sup>

**RESULTS** 

#### **Overall testing results**

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

277 Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)				
Sex						
Female	14 (63.6%)	9 (60%)				
Male	8 (36.4%)	6 (40%)				
Age (years)						
16-24	4 (26.7%)	3 (20%)				

25-34	4 (26.7%)	2 (13.3%(			
35-49	6 (40%)	4 (26.7%)			
>50	8 (36.4%)	6 (40%)			
Ethnic origin					
White	22 (100%)	15 (100%)			

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### 279 Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the 3 patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and 7 were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

298 Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-4) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

305 Regression analysis on confirmed infection

Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03; p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible 309 covariates of confirmed infection.

509 covariates of cor

	People w (seropositive (N=22)	vith confirmed e, any RT-qP		People with acute disease (RT-qPCR reactive and seropositive) (N=15)			
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value	
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029	
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018	
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039	
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032	
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049	
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607	
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825	
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069	

#### 310 Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive,
 irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody
 confirmed positive) among 15 patients respectively.

#### 

#### 315 Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

51 323 

53 324 Correlation between acute and confirmed infection
 54

55325Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI563260.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber57327in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did59328not detect any case with convalescent infection (red curve on Figure 2D).

2 3	329										
4 5	330	Viral clade analysis									
6 7	331	Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and two									
8	332		sequences were not available at the time of analysis. Lineages of SARS-CoV-2 have been identified based on								
9 10	333	-	key amino acid positio		•	-					
11	334		the Spike protein; and c			2			-	<i>.</i>	
12			ost closely related to the	•							
13 14	335		2			` _			0.57	e	e 15 vitai
15	336		e different clades were i		-						
16 17	337 338	Table 3: Gen classification	nomic sequences acces	sed via GIS	SAID listii	ng key ami	ino aci	d locatio	ns used	for SAR	S-CoV-2
18 19	338	Disease	I. Virus Name (GISAID)	EPI ISL #	Date of	Lineage	ORF	ORF3a:	S:614*	N:203**	N:204**
20		Classification Early acute	hCoV-	438032	<b>RT-qPCR</b> 13/03/2020	B(L)	8:84 L	<u>57</u> 0	D	R	G
21			19/Austria/CeMM0191/2020			. ,					_
22 23		Early acute	hCoV- 19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
24		Early acute	hCoV- 19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
25		Early acute	hCoV- 19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
26 27		Early acute	hCoV- 19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
28		Early acute	hCoV- 19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
29		Early acute	hCoV- 19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
30 31		Early acute	hCoV-	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
32		Late acute	19/Austria/CeMM0249/2020 hCoV-	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
33		Late acute	19/Austria/CeMM0267/2020 hCoV-	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
34 35		Late acute	19/Austria/CeMM0276/2020 hCoV-	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
36		Late acute	19/Austria/CeMM0303/2020 hCoV-	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
37 38		Late acute	19/Austria/CeMM0324/2020 hCoV-	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G
30 39			19/Austria/CeMM0337/2020								
40	339	-	ole 3: SARS-CoV-2 cla		-				-		
41 42	340	(GISAID) us	ing specific non-synony	ymous muta	ations in th	e viral gen	ome. C	Clade G is	s define	d by the i	nutations
42 43	341	D614G, C24	1T, C3037T and A2340	3G in the Sp	pike protein	n; and clade	e GR b	y additior	nal RG2	03KR mu	tations in
44	342	the Nucleoca	psid protein N; clade L i	s most close	ely related t	o the Wuha	ın refer	ence strai	n (NC_	045512.2	). <sup>29</sup> Whole
45 46	343	genome data	were available for 13/15	sequences;	data for tw	o sequence	es were	not avail	able at t	he time of	analysis.
40 47	344	Accordingly,	among the 13 sequence	es analysed,	three diffe	rent clades	were i	dentified,	includi	ng clades	L (N=2),
48	345	GR (N=4) ar	nd G (N=7). All three c	lades were	detected in	n early acu	te infe	ction, and	l clade (	G was ad	ditionally
49 50	346	detected in la	ate acute infection. *Fo	r simplicity	reasons, o	nly mutatio	on D61	4G (grey	backgr	ound) in	the Spike
50	347	protein defin	ing clade G is shown.	**Additiona	al mutation	s R203K a	ind G2	04R in th	ne Nucle	eocapsid	orotein N
52	348	•	e GR are also shown in					-		T]	
53 54	349	actining vide		<i>,</i> ,	Spen read						
54 55	579										
56 57	350	DISCUSSIO	N								
58	351	Our results d	lemonstrate that SARS-	CoV-2 RT-	-qPCR test	ing, when	added	to a natio	onal infl	uenza sui	veillance
59 60	352	programme in primary care, can rapidly, early and accurately diagnose COVID-19 during an outbreak. Of the 73									

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and 7 with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains. 

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,<sup>30</sup> we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary as we move away from the first and potentially towards the second COVID-19 wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy. 

Loss of taste of smell has been recognised as an important marker of COVID-19;1 however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.<sup>31</sup> Furthermore, loss of taste could not be objectively confirmed in one third of people<sup>31</sup> suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.<sup>32</sup> Timely and accurate testing is also a prerequisite for effective contact tracing.23 

47 383 

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. No further endemic cases were detected after the

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outbreak. This suggests that combination prevention including rapid testing and case notification in primary care,
 contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge,
 our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza
 screening sites can effectively detect and control a regional outbreak.<sup>18</sup>

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.<sup>33</sup> The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.13

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To our knowledge, this is the first study to show that primary care can contribute to early case detection and termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients, public health, and health systems; nationally and internationally for outbreak epidemiology and control. As countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number  $R_e$  below 1. Austria has increased the number of its sentinels sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.<sup>34</sup> 

Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing.

9 435 

#### 436 CONCLUSIONS

437 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with 438 mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of 439 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 440 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national 441 sentinel surveillance of coronavirus.

Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study. OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay. JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL, MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final version. 

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- 44 457
  457 Ethics approval: The study used secondary anonymised data for which approval was granted by the University
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- 47
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  459 Patient consent for publication: Consent may not be required as no identifiable details on individuals are
  49
  460 reported in this manuscript.
- 51 461 **Patient and public involvement**: No patient involvement.
- 462 Data availability statement: The datasets used and/or analysed during the current study are available from the
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- 56 464 Competing Interests: None declared.
  57

2 3	466	Ref	erences
4 5	467	1.	World Health Organisation (WHO). Clinical management of severe acute respiratory infection when
6 7	468		COVID-19 is suspected. 2020. https://www.who.int/publications-detail/clinical-management-of-severe-
8	469		acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed July 02,
9 10	470		2020).
11	471	2.	Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From
12 13	472		Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020; 172(9): 577-82.
14 15	473	3.	Cheng HY, Jian SW, Liu DP, et al. Contact Tracing Assessment of COVID-19 Transmission Dynamics in
16 17	474		Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. JAMA Intern Med 2020.
18	475	4.	Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in
19 20	476		Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. MMWR
21	477		Morb Mortal Wkly Rep 2020; 69(13): 377-81.
22 23	478	5.	Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect
24 25	479		Dis 2020.
26	480	6.	Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-
27 28	481		2019. <i>Nature</i> 2020; 581(7809): 465-9.
29 30	482	7.	Endo A, Abbott S, Kucharski AJ, et al. Estimating the overdispersion in COVID-19 transmission using
31	483		outbreak sizes outside China. Wellcome Open Res 2020; 5: 67.
32 33	484	8.	Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel
34 35	485		coronavirus (SARS-CoV-2). Science 2020; 368(6490): 489-93.
36 37	486	9.	European Centre for Disease Control and Prevention (ECDC). Rapid Risk Assessment: Coronavirus
38	487		disease 2019 (COVID-19) in the EU/EEA and the UK- ninth update. 2020.
39 40	488		https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-
41	489		19-pandemic-ninth-update (accessed July 02, 2020).
42 43	490	10.	Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a
44	491		modelling study. Lancet Infect Dis 2020.
45 46	492	11.	Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of
47 48	493		Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis 2020; 26(6): 1059-66.
49 50	494	12.	Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in Austria.
51	495		Wien Klin Wochenschr 2020: 1-8.
52 53	496	13.	Popa A, Genger J-W, Nicholson M, et al. Mutational dynamics and transmission properties of SARS-CoV-
54 55	497		2 superspreading events in Austria. bioRxiv 2020: 2020.07.15.204339.
56	498	14.	Independent T. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-
57 58 59	499		hospital-lockdown-latest-a9466281.html. 2020.(Accessed September 05, 2020)
60			

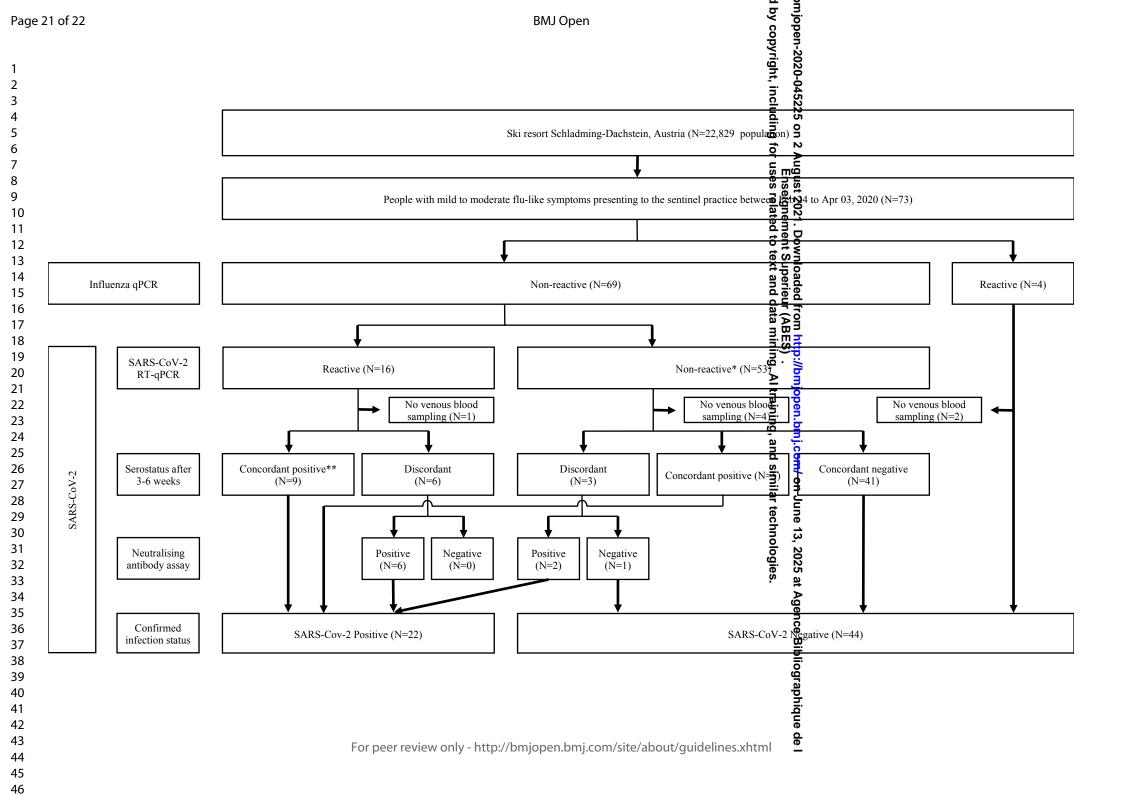
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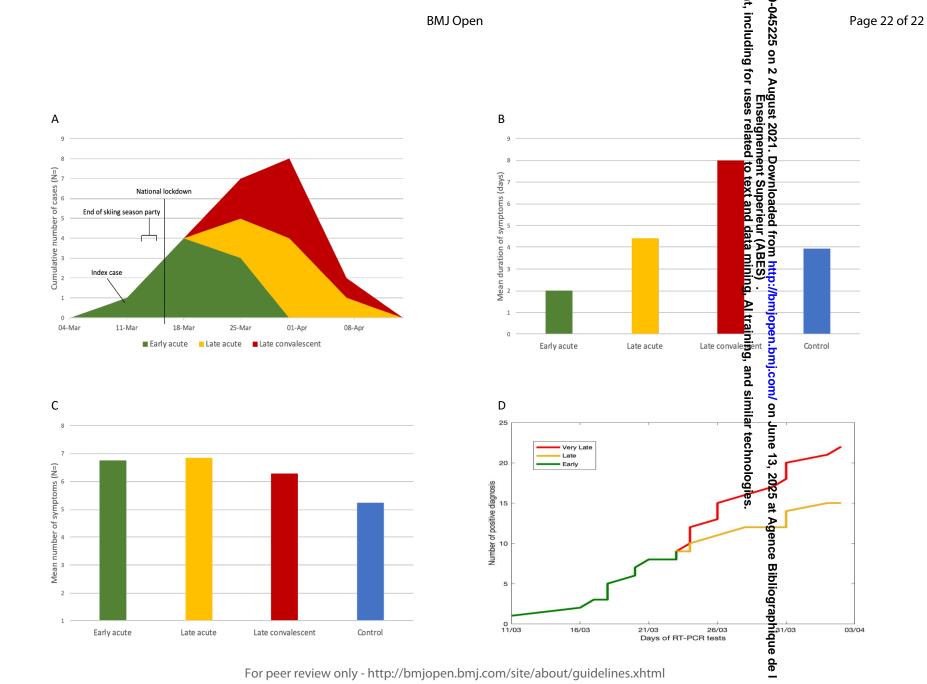
# BMJ Open

2			
3 4	500	15.	(European Centre for Disease Control and Prevention (ECDC). Coronavirus disease 2019 (COVID-19) in
5	501		the EU/EEA and the UK –ninth update, 2020.
6	502		https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-
7 8	503		disease-2019-ninth-update-23-april-2020.pdf (Accessed July 02, 2020)
9 10	504	16.	de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. Eur J
11	505		Gen Pract 2020; 26(1): 58-60.
12 13	506	17.	Hull SA, Williams C, Ashworth M, et al. Suspected COVID-19 in primary care: how GP records
14	507		contribute to understanding differences in prevalence by ethnicity. medRxiv 2020: 2020.05.23.20101741.
15 16	508	18.	European Centres for Disease Control (ECDC). Strategies for the surveillance of COVID-19, 2020.
17 18	509		https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-
19	510		2020.pdf (accessed July 11, 2020).
20 21	511	19.	de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford
22	512		Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-
23 24	513		sectional study. Lancet Infect Dis 2020.
25 26	514	20.	Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk
27	515		Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-
28 29	516		epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (Accessed July 02,
30	517		2020).
31 32	518	21.	Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health
33 34	519		Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020).
35 36	520	22.	Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR.
37	521		https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2 (accessed
38 39	522		September 29, 2020).
40	523	23.	Kretzschmar ME, Rozhnova G, Bootsma MCJ, et al. Impact of delays on effectiveness of contact tracing
41 42	524		strategies for COVID-19: a modelling study. Lancet Public Health 2020.
43 44	525	24.	Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed
45	526		Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020; 57(1):
46 47	527		e100.
48	528	25.	Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with
49 50	529		Acute Respiratory Distress Syndrome in Korea. J Korean Med Sci 2020; 35(14): e149.
51 52	530	26	Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of
53	531	-0.	complex interventions (StaRI): a systematic review and e-Delphi. <i>Implement Sci</i> 2015; 10(1): 42.
54 55	532	27.	Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro
56 57	532	- / •	Surveill 2017; 22(13).
58	534	28	Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Front
59 60	535	20.	Microbiol 2020; 11(1800).

1 2		
3	536	29. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in
4 5	537	genomic epidemiology studies of active hCoV-19 viruses. 2020.
6	538	https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-
7 8	539	genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020).
9 10	540	30. Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening schools,
11	541	the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic
12 13	542	wave in the UK: a modelling study. Lancet Child Adolesc Health 2020.
14	543	31. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in
15 16	544	2013 European Patients With Mild to Moderate COVID-19. Ann Intern Med 2020.
17 18	545	32. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential
19	546	COVID-19. Nat Med 2020.
20 21	547	33. Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper
22 23	548	Airway Symptoms. Ear Nose Throat J 2020: 145561320920762.
24	549	34. NHS England England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020.
25 26	550	2020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-
27 28	551	june-2020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed September 09, 2020).
29	552	
30 31	553	FIGURE LEGENDS
32	333	
33 34	554	FIGURE LEGENDS
35	555	Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15
36 37	556	patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-
38	557	qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing
39 40	558	antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant
41	559	antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44
42	560	patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1
43 44	561	patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with
45 46	562	Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious
46 47	563	mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD
48	564	(N=1, each). ***No concordant negatives.
49 50 51	565	
52	566	Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main
53 54	567	outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing
	001	
55	568	season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last
56		season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)
56 57	568	
56	568 569	endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)

1 2		
3	572	Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive
4 5	573	among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters;
6	574	(C) Mean duration of symptoms; and (D): Mean number of symptoms.
7 8 9 10 11 23 14 15 16 17 18 9 21 22 23 24 25 26 27 28 9 31 23 34 35 37 38 9 40 41 23 44 56 78 9 0 122 34 25 26 78 9 30 31 23 34 56 78 9 40 41 42 44 45 46 78 9 50 51 25 34 55 67 89 60	575	





Page	23	of	22
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		뜻 冷 STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of <i>coffort studies</i> 은 있	
Section/Topic	ltem #	Recommendation	Reported on page
Title and abstract	1	( <i>a</i> ) Indicate the study's design with a commonly used term in the title or the abstract	2
		لله الله الله (b) Provide in the abstract an informative and balanced summary of what was done and what المنافق المعاقية (b) Provide in the abstract an informative and balanced summary of what was done and what was	2,3
Introduction	1	ated	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported 6 2 0	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods	1	and a series	
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure the w-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe sethods of follow-up	5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifies. Get diagnostic criteria, if	6
Data sources/	8*	For each variable of interest, give sources of data and details of methods of assessment (meaurement). Describe	6,7
measurement		comparability of assessment methods if there is more than one group 이수 ㅋ ㅋ ㅋ	
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which rot in the analyses of the second s	5,6,7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	7,8
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	8
		(e) Describe any sensitivity analyses	NA

		BMJ Open BMJ Open 2020	Page 24 o
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, exagnined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information to me posures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest     Indicate number of participants with missing data for each variable of interest       (c) Summarise follow-up time (eg, average and total amount)     Indicate numbers of outcome events or summary measures over time	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their pred to be a confidence	9,10
		interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningf 通册 period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations		ning n.b	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information		ar tur	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in controls in case-control studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published exan bles of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine 🛱 rg/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.spide-statement.org.

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## Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in Austria: Evidence of effective sentinel surveillance screening in primary care (REAP-1)

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<b>Primary Subject Heading</b> :	General practice / Family practice

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Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	PRIMARY CARE, COVID-19, Public health < INFECTIOUS DISEASES, VIROLOGY
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3 4	1	Title: Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in
5 6	2	Austria: Evidence of effective sentinel surveillance screening in primary care (REAP-1)
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34 35	48	
36 37	49	ABSTRACT
38	50	<b>Objectives:</b> We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 40	51	regional COVID-19 outbreak in Austria.
41 42	52	Design: Prospective cohort study.
43 44	53	Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.
45	54	Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 47	55	April, 2020.
48 49	56	Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
50 51	57	chain reaction (RT-qPCR).
52	58	Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the
53 54	59	outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
54 55	60	with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
56	61	convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
57 58	62	accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.
58 59 60	02	accuracy of R1-q1 CR, the duration and variety of symptoms, and the number of viral clades present.

#### **BMJ** Open

**Results:** Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent),
44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all
acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of
symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days
(2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was
associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
 surveillance of coronavirus.

74 Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people testing RT-qPCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

#### 84 INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.<sup>1</sup> In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,<sup>2</sup> infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that.<sup>3,4</sup> and gradually declining over 7-10 days.<sup>5,6 7</sup> 

95 SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a
96 large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may
97 cause up to 80% of secondary transmissions.<sup>8</sup> Although symptomatic infection is common (17 %, range 4-41%),
98 the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.<sup>9-11</sup>
99 Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all
100 documented infections.<sup>12</sup> Superspreading events have been reported from across the globe, and countries

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101 achieving early viral suppression took rapid and decisive action to implement comprehensive case identification 102 and testing, combined with contact tracing and isolation.<sup>13,14</sup> For epidemic control of COVID-19, the effective 103 reproduction number,  $R_e$ , needs to be less than 1; the presence of undetected and persistent infection within the 104 population, even if very small, can increase  $R_e$  and induce a secondary peak of infections. Therefore, rapid 105 identification and containment of infection is a key factor for the prevention of onward transmission and 106 controlling the virus to protect the public.<sup>15</sup>

12 107 

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.<sup>16</sup> Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.<sup>16,17</sup> Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and via mobile units.<sup>18</sup> The first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512 cases and 7.389 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.<sup>19-21</sup> The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."22 However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.<sup>23</sup> In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.<sup>24</sup> The new service supplemented the existing national health hotline for people at risk of COVID-19.25 RT-gPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.26 Early detection of SARS-CoV-2 is essential for effective contact tracing,<sup>27</sup> and whole genome sequencing may provide data on dynamics of transmission. 17,28 

The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak. 

140       METHODS         141       Setting         142       This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort         143       of Schladming-Dachstein, political subdistrict of Greebming (population 22,829). Austria, The study was         144       conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by         145       RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation         146       of the national lockdown policy on March 16, which led to premature termination of the first cases in Austria,         147       people with flu-like symptoms were advised to call the national health hotine instead of directly presenting to the         148       hoospital or GP. Patients were indvised to phone the GP or receive in-home testing by mobile testing units, and         149       home self-isolate and self-care. Asymptomatic people were excluded from this study.         151       Design         152       Design         153       We conducted a longitudinal evaluation comprising a prospective cohort to examine the inpact of SARS-Cov-2         154       RT-qPCR teasing on COVID-19 case detection. Retween February 24 and April 03, 2020, RT-qPCR teasing and seropositivity data were collected to compare two groups within this cohort of patients:         155       • Patients testing RT-qPCR reactive at presentation with acuted is	2 3	139	
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144       conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by         145       RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation         146       of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients         147       presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria,         148       people with flu-like symptoms were advised to phone the GP or receive in-home testing by mobile testing units, and         151       hopsital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and         152       Design         153       We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2         154       RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and         155       • Patients testing RT-qPCR reactive at presentation with acute disease         156       • Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).         158       We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR         159       nad positive serostatus; and confirmed infection as the presence of convalescent phase (confirmed nafuenza)         161       Intervention       <	10		
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<ul> <li>appointments for britts-cov-2 K1-qr excreasing. K1-qr excreasing were available within 24 hours, and those</li> <li>patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a</li> <li>minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the</li> <li>local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat</li> <li>RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2</li> <li>infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number</li> <li>of days from the first patient to the last patient testing RT-qPCR reactive at the GP.</li> </ul>	47		
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<ul> <li>55</li> <li>56</li> <li>57</li> <li>57</li> <li>57</li> <li>58</li> <li>59</li> <li>173</li> </ul>		169	local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat
<ul> <li>56 1/1 Infection using ELISA IgG and neutralizing antibody assay. we defined the period of the outbreak as the number</li> <li>57 172 of days from the first patient to the last patient testing RT-qPCR reactive at the GP.</li> <li>58</li> <li>59 173</li> </ul>		170	RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2
<ul> <li>57 172 of days from the first patient to the last patient testing RT-qPCR reactive at the GP.</li> <li>58</li> <li>59 173</li> </ul>		171	infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number
59 173	57	172	of days from the first patient to the last patient testing RT-qPCR reactive at the GP.
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Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year, participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country.

180 Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

24 187

188 Testing

#### 189 RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).<sup>26</sup> RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection. 

36 195 Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),<sup>29</sup> and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.<sup>30</sup> Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of SARS-CoV-2. Reagent wells of the EDI<sup>TM</sup> Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA, 

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IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI). Neutralising antibody assay Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers  $\geq 10$  were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.<sup>31</sup> Outcome measures and statistical analysis We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following four testing, viral and genomic outcomes: Outcome A: The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative. To determine the accuracy of RT-qPCR, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive). **Outcome B:** The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters). We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) vs. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent). Outcome C: The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent sero-positivity (confirmed infection) to determine any potential correlation between these stages of disease. We used multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

249 positive) and all patients with confirmed infection by calculating the correlation coefficient r, and estimating the 250 95% CI.

**Outcome D**: The number of viral clades implicated in the outbreak. To do this, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.<sup>17,28</sup> The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).<sup>32</sup> Sequences were aligned in MEGA7 and nonsynonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.<sup>33</sup>

#### **RESULTS**

#### **Overall testing results**

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

275 Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)		
Sex				
Female	14 (63.6%)	9 (60%)		
Male	8 (36.4%)	6 (40%)		
Age (years)				
16-24	4 (26.7%)	3 (20%)		

		]		
25-34	4 (26.7%)	2 (13.3%(		
35-49	6 (40%)	4 (26.7%)		
>50	8 (36.4%)	6 (40%)		
Ethnic origin				
White	22 (100%)	15 (100%)		

## 277 Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

296 Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

303 Regression analysis on confirmed infection
56

Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03; p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible covariates of confirmed infection.

507 covariates of com

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)			
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value	
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029	
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018	
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039	
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032	
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049	
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607	
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825	
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069	

#### 308 Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive,
 irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody
 confirmed positive) among 15 patients respectively.

#### 37 312

#### 313 Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

51 <u>321</u> 

# 53 322 Correlation between acute and confirmed infection 54

55323Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI563240.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber58325in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did59326not detect any case with convalescent infection (red curve on Figure 2D).

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2											
3 4	327										
5 6	328	Viral clade a	analysis								
7	329	Thirteen of 1	5 full-length genome se	equences w	ere availab	le for clade	e analy	sis via G	ISAID	(Table 3)	); and two
8 9	330	sequences we	ere not available at the	time of ana	alysis. Line	ages of SA	RS-Co	V-2 hav	e been i	identified	l based on
10	331	mutations in	key amino acid positio	ons.33 Clad	e G is defi	ned by the	e mutat	tions D6	14G, C2	241T, C3	8037T and
11	332	A23403G in	the Spike protein; and c	lade GR by	additional	RG203KR	t mutat	ions in th	ne Nucle	eocapsid	protein N;
12 13	333	clade L is mo	ost closely related to the	Wuhan ref	erence stra	in (NC_045	5512.2)	. <sup>34</sup> Accor	dingly,	among tl	he 13 viral
14	334	isolates, three	e different clades were i	dentified, ir	ncluding cla	ade L (N=2	), GR (	N=4) and	d L (N=	7).	
15 16	225	Table 2. Ca	•			· · · · · · · · · · · ·	•		` 		
17	335		nomic sequences acces	sed via GI	SAID listi	ig key ami	no aci	u locatio	ns used	I IOF SAI	KS-COV-2
18 19	336	classification	1. Virus Name (GISAID)	EPI ISL #	Date of	Lineage	ORF	ORF3a:	S:614*	N:203**	N:204**
20		Classification	· ·		RT-qPCR	0	<u>8: 84</u>	<u>57</u>			
21		Early acute	hCoV- 19/Austria/CeMM0191/2020	438032	13/03/2020	B(L)	L	Q	D	R	G
22 23		Early acute	hCoV- 19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
24		Early acute	hCoV- 19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
25		Early acute	hCoV- 19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
26 27		Early acute	hCoV- 19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
28		Early acute	hCoV- 19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	К	R
29 30		Early acute	hCoV- 19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
30 31		Early acute	hCoV- 19/Austria/CeMM0249/2020	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
32		Late acute	hCoV-	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
33 34		Late acute	19/Austria/CeMM0267/2020 hCoV-	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
35		Late acute	19/Austria/CeMM0276/2020 hCoV-	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
36 27		Late acute	19/Austria/CeMM0303/2020 hCoV-	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
37 38		Late acute	19/Austria/CeMM0324/2020 hCoV-	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G
39	337	Cantion Tal	19/Austria/CeMM0337/2020 ble 3: SARS-CoV-2 cla	l des are cla	ssified by	The Globa	 1 Initia	tive on S	haring	 All Influ	enza Data
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42	339	· /	1T, C3037T and A2340	·		e				2	
43 44	340		psid protein N; clade L i				-				
44 45	341		were available for 13/15		•				. –		
46	342	•	among the 13 sequence	-		-					•
47 48	343	•••	antoing the 15 sequence $d \in G$ (N=7). All three c	•						-	
49	343	× /	~ /			2					2
50			ate acute infection. *For			•			-	<i>.</i>	•
51 52	345	-	ing clade G is shown.				ina G2	04K in th	ie Nucl	eocapsid	protein N
53	346	defining clad	e GR are also shown in	grey. ORF,	open read	ng frame.					
54 55	347										
55 56 57	348	DISCUSSIO	N								
58	349	Our results d	lemonstrate that SARS-	CoV-2 RT	-qPCR test	ing, when a	added	to a natio	onal inf	luenza su	irveillance
59 60	350	programme i	n primary care, can rapi	dly, early a	nd accurate	ly diagnose	e COV	ID-19 du	ring an o	outbreak.	Of the 73

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains. 

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,<sup>35</sup> we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy. 

Loss of taste of smell has been recognised as an important marker of COVID-19;36.37 however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.<sup>38</sup> Furthermore, loss of taste could not be objectively confirmed in one third of people<sup>38</sup> suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.<sup>39</sup> Timely and accurate testing is also a prerequisite for effective contact tracing.27 

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The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or

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did not attend the practice because of mild disease.<sup>40</sup> No further endemic cases were detected after the outbreak.
 This suggests that combination prevention including rapid testing and case notification in primary care, contact
 tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study
 is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening
 sites can effectively detect and control a regional outbreak.<sup>22</sup>

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.<sup>41</sup> However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),42,43 but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,<sup>10</sup> asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.28

To our knowledge, this is the first study to show that primary care can contribute to early case detection and
termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients,
public health, and health systems; nationally and internationally for outbreak epidemiology and control. As

countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number  $R_e$  below 1. Austria has increased the number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.<sup>44</sup> Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2 lateral flow antigen testing.

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#### 441 CONCLUSIONS

442 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with 443 mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of 444 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 445 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national 446 sentinel surveillance of coronavirus.

448 Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study.
449 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
450 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
451 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
452 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
453 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
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47 458 administration. We thank the patients of Schladming-Dachstein for participating in the study.

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54 462 Ethics approval: The study used secondary anonymised data for which approval was granted by the University
55 463 of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).

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2 3	466	Patient and public involvement: No patient involvement.
4	400	Patient and public involvement. No patient involvement.
5	467	Data availability statement: The datasets used and/or analysed during the current study are available from the
6 7	468	corresponding author on reasonable request.
8 9	469	Competing Interests: None declared.
10 11	470	
12 13	471	References
14	472	1. World Health Organisation (WHO). Clinical management of severe acute respiratory infection when
15	473	COVID-19 is suspected. 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-
16	474	respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed July 02, 2020).
17 18	475 476	2. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020; 172(9): 577-82.
19 20	477	3. Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT, Lin HH. Contact Tracing Assessment of COVID-19
20 21 22	478 479	Transmission Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. JAMA Intern Med 2020.
23	480	4. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections
24	481	in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. MMWR
25	482	Morb Mortal Wkly Rep 2020; 69(13): 377-81.
26	483	5. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin
27	484	Infect Dis 2020.
28		
29 30	485 486	6. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581(7809): 465-9.
30 31		
32	487 488	7. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-
33	488 489	CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta- analysis. medRxiv 2020: 2020.07.25.20162107.
34	490	8. Endo A, Abbott S, Kucharski AJ, Funk S. Estimating the overdispersion in COVID-19 transmission
35 36	490 491	using outbreak sizes outside China. Wellcome Open Res 2020; 5: 67.
37	492	9. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus
38	493	symptomatic COVID-19. Lancet 2021; 397(10269): 93-4.
39	494	10. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of
40	495	asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis.
41 42	496	Official Journal of the Association of Medical Microbiology and Infectious Disease Canada 2020; 5(4): 223-34.
42 43	497	11. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their
43 44	498	close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis 2020.
45	499	12. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of
46	500	novel coronavirus (SARS-CoV-2). Science 2020; 368(6490): 489-93.
47		
48	501	13. European Centre for Disease Control and Prevention (ECDC). Rapid Risk Assessment: Coronavirus
49	502 503	disease 2019 (COVID-19) in the EU/EEA and the UK- ninth update. 2020. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-19-
50	503 504	pandemic-ninth-update (accessed July 02, 2020).
51		
52 53	505 506	14. Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. Lancet Infect Dis 2020.
54 57	507	15. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of
55 56	508	Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis 2020; 26(6): 1059-66.
50 57	509	16. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in
58	510	Austria. Wien Klin Wochenschr 2020: 1-8.
59	511	17. Popa A, Genger JW, Nicholson MD, et al. Genomic epidemiology of superspreading events in Austria
60	512	reveals mutational dynamics and transmission properties of SARS-CoV-2. Sci Transl Med 2020; 12(573).

18. Independent T. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-hospital-lockdown-latest-a9466281.html. 2020.(Accessed September 05, 2020) (European Centre for Disease Control and Prevention (ECDC), Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK -ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-ninth-update-23-april-2020.pdf (Accessed July 02, 2020) 20. de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. Eur J Gen Pract 2020; 26(1): 58-60. Hull SA, Williams C, Ashworth M, Carvalho C, Boomla K. Prevalence of suspected COVID-19 21. infection in patients from ethnic minority populations: a cross-sectional study in primary care. British Journal of General Practice 2020; 70(699): e696-e704. European Centres for Disease Control (ECDC). Strategies for the surveillance of COVID-19, 2020. 22. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020). de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford 23. Royal College of General Practitioners Research and Surveillance Centre primary care network; a cross-sectional study. Lancet Infect Dis 2020. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk 24. Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (Accessed July 02, 2020). 25. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020). 26. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c 2 (accessed September 29, 2020). 27. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. The Lancet Public Health. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed 28. Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020; 57(1): e100. 29. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. J Korean Med Sci 2020; 35(14): e149. 30. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of complex interventions (StaRI): a systematic review and e-Delphi. Implementation Science 2015; 10(1): 42. 31. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Eurosurveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2017; 22(13). Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Frontiers 32. in Microbiology 2020; 11(1800). 33. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statementsclarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020). Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening 34. schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. The Lancet Child & Adolescent Health. Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The Association of "Loss of 35. Smell" to COVID-19: A Systematic Review and Meta-Nnalysis. Am J Med Sci 2020. von Bartheld CS, Hagen MM, Butowt R. Prevalence of Chemosensory Dysfunction in COVID-19 36. Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. ACS Chem Neurosci 2020; 11(19): 2944-61.

564 37. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste
 565 in 2013 European Patients With Mild to Moderate COVID-19. Ann Intern Med 2020.

Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict
 potential COVID-19. Nat Med 2020.

Maltezou HC, Vorou R, Papadima K, et al. Transmission dynamics of SARS-CoV-2 within families
with children in Greece: A study of 23 clusters. J Med Virol 2020.

57040.Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper571Airway Symptoms. Ear Nose Throat J 2020: 145561320920762.

57241.Sung Y-T, Wu J-S. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-573RRP): A new technique for psychological measurement. Behav Res Methods 2018; 50(4): 1694-715.

574 42. Rojas-Lechuga MJ, Izquierdo-Domínguez A, Chiesa-Estomba C, et al. Chemosensory dysfunction in
 575 COVID-19 out-patients. Eur Arch Otorhinolaryngol 2020: 1-8.

57643.NHS England England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020.5772020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-5782020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed September 09, 2020).

#### 580 FIGURE LEGENDS

581 Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15 582 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-

<sup>7</sup> 583 gPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing

584 antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant

585 antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44

586 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1

patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with

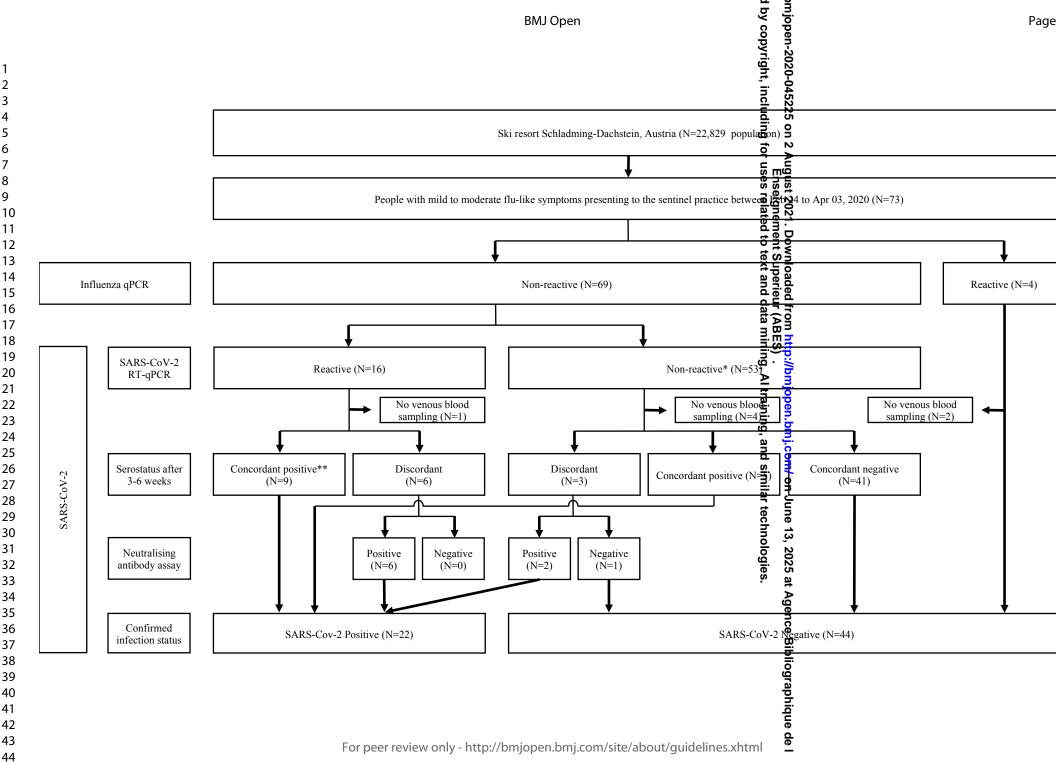
588 Influenza. Antibody status was not available for 7 patients. **\*\***Final clinical diagnoses included infectious

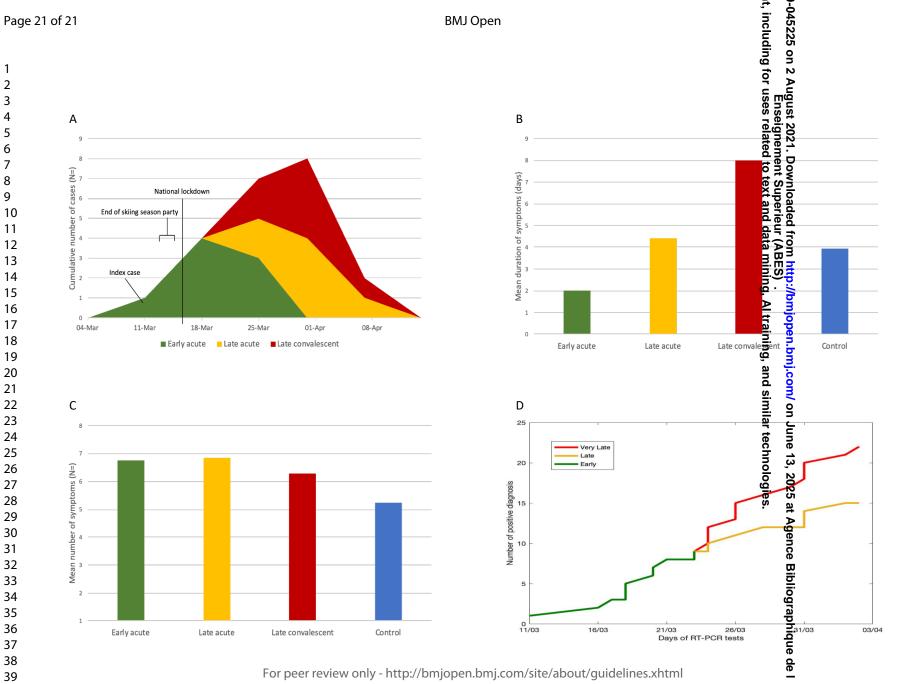
6 589 mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD

 $\frac{7}{2}$  590 (N=1, each). \*\*\*No concordant negatives.

 Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive) in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B) Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms. 

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		STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cofficient studies 도 있	
Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction		ate i 121	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods		and a serie of the	
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure to be and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe fiethods of follow-up	5
		(b) For matched studies, give matching criteria and number of exposed and unexposed 🛓	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifies. Get diagnostic criteria, if	7,8
Data sources/	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe	6,7
measurement		comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	6,13
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which are bound by why	7,8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	7,8
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	8
		(e) Describe any sensitivity analyses	NA
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, exagining for eligibility, confirmed	8
		eligible, included in the study, completing follow-up, and analysed       Image: Completing follow-up, and analysed         (b) Give reasons for non-participation at each stage       Image: Completing follow-up, and analysed	8
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information for the posures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	( <i>a</i> ) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their pre to be a structure of the stru	9,10
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaning full period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations		ning b	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of malyses, results from similar studies, and other relevant evidence	12,13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information		ar t	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in controls in case-control studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published exam bles of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine 🛱 rg/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.spide-statement.org.

## Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (REAP-1)

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<b>Primary Subject Heading</b> :	General practice / Family practice

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Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	PRIMARY CARE, COVID-19, Public health < INFECTIOUS DISEASES, VIROLOGY
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# BMJ Open

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3 4	1	Title: Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A
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34 35	48	
36 37	49	ABSTRACT
38	50	<b>Objectives:</b> We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 40	51	regional COVID-19 outbreak in Austria.
41 42	52	Design: Prospective cohort study.
43 44	53	Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.
45	54	Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 47	55	April, 2020.
48 49	56	Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
50 51	57	chain reaction (RT-qPCR).
52	58	Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the
53 54	59	outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
54 55	60	with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
56	61	convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
57 58	62	accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.
58 59 60	02	accuracy of R1-q1 CR, the duration and variety of symptoms, and the number of viral clades present.

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**Results:** Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent),
44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all
acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of
symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days
(2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was
associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
 surveillance of coronavirus.

74 Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people testing RT-qPCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

#### 84 INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.<sup>1</sup> In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,<sup>2</sup> infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that.<sup>3,4</sup> and gradually declining over 7-10 days.<sup>5,6 7</sup> 

95 SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a
96 large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may
97 cause up to 80% of secondary transmissions.<sup>8</sup> Although symptomatic infection is common (17 %, range 4-41%),
98 the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.<sup>9-11</sup>
99 Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all
100 documented infections.<sup>12</sup> Superspreading events have been reported from across the globe, and countries

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101 achieving early viral suppression took rapid and decisive action to implement comprehensive case identification 102 and testing, combined with contact tracing and isolation.<sup>13,14</sup> For epidemic control of COVID-19, the effective 103 reproduction number,  $R_e$ , needs to be less than 1; the presence of undetected and persistent infection within the 104 population, even if very small, can increase  $R_e$  and induce a secondary peak of infections. Therefore, rapid 105 identification and containment of infection is a key factor for the prevention of onward transmission and 106 controlling the virus to protect the public.<sup>15</sup>

12 107 

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.<sup>16</sup> Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.<sup>16,17</sup> Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and via mobile units.<sup>18</sup> The first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512 cases and 7.389 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.<sup>19-21</sup> The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."22 However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.<sup>23</sup> In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.<sup>24</sup> The new service supplemented the existing national health hotline for people at risk of COVID-19.25 RT-gPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.26 Early detection of SARS-CoV-2 is essential for effective contact tracing,<sup>27</sup> and whole genome sequencing may provide data on dynamics of transmission.<sup>17,28</sup> 

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The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak. 

2 3	139	
4 5	140	METHODS
6 7	141	Setting
8 9	142	This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort
10	143	of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was
11 12	144	conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by
13	145	RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation
14 15	146	of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients
16	147	presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria,
17 18	148	people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the
19	149	hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and
20 21	150	home self-isolate and self-care. Asymptomatic people were excluded from this study.
22	151	
23 24		Design
25	152	Design
26 27	153	We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2
28	154	RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and
29 30	155	seropositivity data were collected to compare two groups within this cohort of patients:
31	156	• Patients testing RT-qPCR reactive at presentation with acute disease
32 33	157	<ul> <li>Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).</li> </ul>
33 34		
35	158	We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR
36 37	159	and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6
38	160	weeks after the acute illness, irrespective of the RT-qPCR result.
39 40	161	
41 42	162	Intervention
42 43	162	On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza
44	163	
45 46	164	Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.
47	165	Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day
48 49	166	appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those
50	167	patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a
51 52	168	minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the
53	169	local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat
54	170	RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2
55 56	171	infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number
57	172	of days from the first patient to the last patient testing RT-qPCR reactive at the GP.
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Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year, participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country.

180 Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

24 187

188 Testing

#### 189 RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).<sup>26</sup> RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection. 

36 195 Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),<sup>29</sup> and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.<sup>30</sup> Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of SARS-CoV-2. Reagent wells of the EDI<sup>TM</sup> Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA, 

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IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI). Neutralising antibody assay Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers  $\geq 10$  were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.<sup>31</sup> Outcome measures and statistical analysis We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following four testing, viral and genomic outcomes: Outcome A: The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative. To determine the accuracy of RT-qPCR, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive). **Outcome B:** The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters). We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) vs. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent). Outcome C: The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent sero-positivity (confirmed infection) to determine any potential correlation between these stages of disease. We used multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

249 positive) and all patients with confirmed infection by calculating the correlation coefficient r, and estimating the 250 95% CI.

**Outcome D**: The number of viral clades implicated in the outbreak. To do this, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.<sup>17,28</sup> The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).<sup>32</sup> Sequences were aligned in MEGA7 and nonsynonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.<sup>33</sup>

#### **RESULTS**

#### **Overall testing results**

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

275 Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)				
Sex						
Female	14 (63.6%)	9 (60%)				
Male	8 (36.4%)	6 (40%)				
Age (years)						
16-24	4 (26.7%)	3 (20%)				

		]
25-34	4 (26.7%)	2 (13.3%(
35-49	6 (40%)	4 (26.7%)
>50	8 (36.4%)	6 (40%)
Ethnic origin		
White	22 (100%)	15 (100%)

## 277 Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

296 Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

303 Regression analysis on confirmed infection
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Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03; p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible covariates of confirmed infection.

507 covariates of com

	People w (seropositive (N=22)	vith confirmed e, any RT-qP		People with acute disease (RT-qPCR reactive and seropositive) (N=15)			
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value	
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029	
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018	
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039	
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032	
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049	
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607	
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825	
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069	

#### 308 Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive,
 irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody
 confirmed positive) among 15 patients respectively.

#### 37 312

#### 313 Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

51 <u>321</u> 

# 53 322 Correlation between acute and confirmed infection 54

55323Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI563240.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber58325in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did59326not detect any case with convalescent infection (red curve on Figure 2D).

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2 3	227										
4	327										
5 6	328	Viral clade a	analysis								
7	329	Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and two									
8 9	330	sequences w	ere not available at the	time of an	alysis. Line	ages of SA	RS-Co	oV-2 hav	e been	identified	l based on
9 10	331	mutations in	key amino acid position	ons.33 Clac	le G is defi	ned by the	e mutat	tions D6	14G, C2	241T, C3	037T and
11	332	A23403G in	the Spike protein; and c	lade GR b	y additional	RG203KR	t mutat	ions in th	ne Nucle	eocapsid	protein N;
12 13	333	clade L is mo	ost closely related to the	Wuhan re	ference stra	in (NC_045	5512.2)	. <sup>34</sup> Accor	dingly,	among tl	ne 13 viral
14	334	isolates, three	e different clades were i	dentified, i	ncluding cla	ade L (N=2	), GR (	(N=4) and	d L (N=	7).	
15 16	335	Table 3. Ca	nomic sequences acces	and via Cl				dlaatia	<b>n</b> a <b>n</b> aad	I for CAT	DE CoV 2
17	335 336	classification	-	seu via Gi	ISAID IISUI	ig key ann	no aci	u locatio	ns used	I IOF SAI	XS-COV-2
18 19	330	Disease	I. Virus Name (GISAID)	EPI ISL #	Date of	Lineage	ORF	ORF3a:	S:614*	N:203**	N:204**
20		Classification Early acute	hCoV-	438032	RT-qPCR 13/03/2020	B(L)	<u>8: 84</u>	<u>57</u> Q	D	R	G
21		Early acute	19/Austria/CeMM0191/2020 hCoV-	438078	21/03/2020	B(L)	L	Q	D	R	G
22 23			19/Austria/CeMM0248/2020	6							
24		Early acute	hCoV- 19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
25 26		Early acute	hCoV- 19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
20 27		Early acute	hCoV- 19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
28		Early acute	hCoV- 19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
29 30		Early acute	hCoV- 19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
31		Early acute	hCoV- 19/Austria/CeMM0249/2020	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
32		Late acute	hCoV- 19/Austria/CeMM0267/2020	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
33 34		Late acute	hCoV- 19/Austria/CeMM0276/2020	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
35		Late acute	hCoV-	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
36 37		Late acute	19/Austria/CeMM0303/2020 hCoV-	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
38		Late acute	19/Austria/CeMM0324/2020 hCoV-	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G
39	337	Caption Tal	19/Austria/CeMM0337/2020 ble 3: SARS-CoV-2 cla	des are cl	assified by	 The Global	   Initia	tive on S	haring	All Influ	enza Data
40 41	338	-	ing specific non-synon		-				-		
42	339	· /	<b>U</b> 1 <b>U</b> 1	, ,		U				2	
43 44	340	D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC 045512.2). <sup>34</sup> Whole									
45	341		• • ·		•				. –		·
46	342	genome data were available for 13/15 sequences; data for two sequences were not available at the time of analysis. Accordingly, among the 13 sequences analysed, three different clades were identified, including clades L (N=2),									
47 48	343		and G (N=7). All three $c$	•						-	
49	344		ate acute infection. *For			•					•
50 51	345		ing clade G is shown.			•			-		-
52		-	-				in <b>u</b> 02	04K III U	le Inuci	eocapsiu	protein N
53	346	defining clad	e GR are also shown in	grey. ORF	, open readi	ng frame.					
54 55	347										
56 57	348	DISCUSSIC	N								
58	349	Our results demonstrate that SARS-CoV-2 RT-qPCR testing, when added to a national influenza surveillance					irveillance				
59	350	programme in primary care, can rapidly, early and accurately diagnose COVID-19 during an outbreak. Of the 73									
60		-		-		-			-		

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains. 

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,<sup>35</sup> we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy. 

Loss of taste of smell has been recognised as an important marker of COVID-19;36.37 however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.<sup>38</sup> Furthermore, loss of taste could not be objectively confirmed in one third of people<sup>38</sup> suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.<sup>39</sup> Timely and accurate testing is also a prerequisite for effective contact tracing.27 

46 380

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or

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#### **BMJ** Open

did not attend the practice because of mild disease.<sup>40</sup> No further endemic cases were detected after the outbreak.
 This suggests that combination prevention including rapid testing and case notification in primary care, contact
 tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study
 is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening
 sites can effectively detect and control a regional outbreak.<sup>22</sup>

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.<sup>41</sup> However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),42,43 but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,<sup>10</sup> asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.28

To our knowledge, this is the first study to show that primary care can contribute to early case detection and
termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients,
public health, and health systems; nationally and internationally for outbreak epidemiology and control. As

countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number  $R_e$  below 1. Austria has increased the number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.<sup>44</sup> Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2 lateral flow antigen testing.

#### 441 CONCLUSIONS

442 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with 443 mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of 444 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 445 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national 446 sentinel surveillance of coronavirus.

448 Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study.
449 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
450 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
451 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
452 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
453 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
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54 462 Ethics approval: The study used secondary anonymised data for which approval was granted by the University
55 463 of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).

**Patient consent for publication:** Verbal consent was received from patients for study participation.

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2 3	466	Patient and public involvement: No patient involvement.
4	400	<b>Patient and public involvement</b> . No patient involvement.
5	467	Data availability statement: The datasets used and/or analysed during the current study are available from the
6 7	468	corresponding author on reasonable request.
8 9	469	Competing Interests: None declared.
10 11	470	
12 13	471	References
14	472	1. World Health Organisation (WHO). Clinical management of severe acute respiratory infection when
15	473	COVID-19 is suspected. 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-
16	474	respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed July 02, 2020).
17 18	475 476	2. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020; 172(9): 577-82.
19 20	477	3. Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT, Lin HH. Contact Tracing Assessment of COVID-19
20 21 22	478 479	Transmission Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. JAMA Intern Med 2020.
23	480	4. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections
24	481	in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. MMWR
25	482	Morb Mortal Wkly Rep 2020; 69(13): 377-81.
26	483	5. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin
27	484	Infect Dis 2020.
28		
29	485 486	6. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581(7809): 465-9.
30 21		
31 32	487	7. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-
33	488 489	CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta- analysis. medRxiv 2020: 2020.07.25.20162107.
34 35	490	8. Endo A, Abbott S, Kucharski AJ, Funk S. Estimating the overdispersion in COVID-19 transmission
36	491	using outbreak sizes outside China. Wellcome Open Res 2020; 5: 67.
37	492	9. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus
38	493	symptomatic COVID-19. Lancet 2021; 397(10269): 93-4.
39	494	10. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of
40	495	asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis.
41	496	Official Journal of the Association of Medical Microbiology and Infectious Disease Canada 2020; 5(4): 223-34.
42		
43 44	497 498	11. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis 2020.
44 45		
46	499	12. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of
47	500	novel coronavirus (SARS-CoV-2). Science 2020; 368(6490): 489-93.
48	501	13. European Centre for Disease Control and Prevention (ECDC). Rapid Risk Assessment: Coronavirus
49	502	disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update. 2020.
50	503 504	https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-19- pandemic-ninth-update (accessed July 02, 2020).
51		
52	505	14. Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore:
53	506	a modelling study. Lancet Infect Dis 2020.
54	507	15. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of
55 56	508	Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis 2020; 26(6): 1059-66.
56 57	509	16. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in
57	510	Austria. Wien Klin Wochenschr 2020: 1-8.
50 59	511	17. Popa A, Genger JW, Nicholson MD, et al. Genomic epidemiology of superspreading events in Austria
60	512	reveals mutational dynamics and transmission properties of SARS-CoV-2. Sci Transl Med 2020; 12(573).

18. Independent T. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-hospital-lockdown-latest-a9466281.html. 2020.(Accessed September 05, 2020) (European Centre for Disease Control and Prevention (ECDC), Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK -ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-ninth-update-23-april-2020.pdf (Accessed July 02, 2020) 20. de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. Eur J Gen Pract 2020; 26(1): 58-60. Hull SA, Williams C, Ashworth M, Carvalho C, Boomla K. Prevalence of suspected COVID-19 21. infection in patients from ethnic minority populations: a cross-sectional study in primary care. British Journal of General Practice 2020; 70(699): e696-e704. European Centres for Disease Control (ECDC). Strategies for the surveillance of COVID-19, 2020. 22. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020). de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford 23. Royal College of General Practitioners Research and Surveillance Centre primary care network; a cross-sectional study. Lancet Infect Dis 2020. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk 24. Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (Accessed July 02, 2020). 25. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020). 26. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c 2 (accessed September 29, 2020). 27. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. The Lancet Public Health. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed 28. Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020; 57(1): e100. 29. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. J Korean Med Sci 2020; 35(14): e149. 30. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of complex interventions (StaRI): a systematic review and e-Delphi. Implementation Science 2015; 10(1): 42. 31. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Eurosurveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2017; 22(13). Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Frontiers 32. in Microbiology 2020; 11(1800). 33. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statementsclarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020). Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening 34. schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. The Lancet Child & Adolescent Health. Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The Association of "Loss of 35. Smell" to COVID-19: A Systematic Review and Meta-Nnalysis. Am J Med Sci 2020. von Bartheld CS, Hagen MM, Butowt R. Prevalence of Chemosensory Dysfunction in COVID-19 36. Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. ACS Chem Neurosci 2020; 11(19): 2944-61.

564 37. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste
 565 in 2013 European Patients With Mild to Moderate COVID-19. Ann Intern Med 2020.

Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict
 potential COVID-19. Nat Med 2020.

Maltezou HC, Vorou R, Papadima K, et al. Transmission dynamics of SARS-CoV-2 within families
with children in Greece: A study of 23 clusters. J Med Virol 2020.

57040.Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper571Airway Symptoms. Ear Nose Throat J 2020: 145561320920762.

57241.Sung Y-T, Wu J-S. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-573RRP): A new technique for psychological measurement. Behav Res Methods 2018; 50(4): 1694-715.

574 42. Rojas-Lechuga MJ, Izquierdo-Domínguez A, Chiesa-Estomba C, et al. Chemosensory dysfunction in
 575 COVID-19 out-patients. Eur Arch Otorhinolaryngol 2020: 1-8.

57643.NHS England England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020.5772020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-5782020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed September 09, 2020).

#### 580 FIGURE LEGENDS

581 Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15 582 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-

<sup>7</sup> 583 gPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing

584 antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant

585 antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44

586 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1

patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with

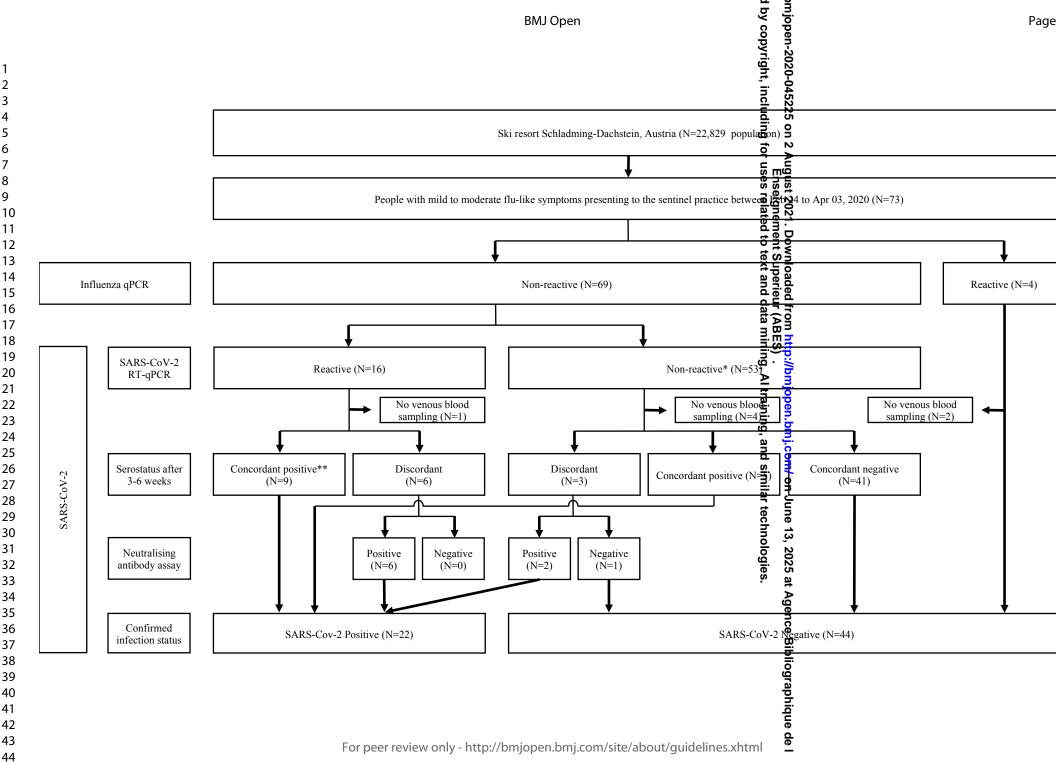
588 Influenza. Antibody status was not available for 7 patients. **\*\***Final clinical diagnoses included infectious

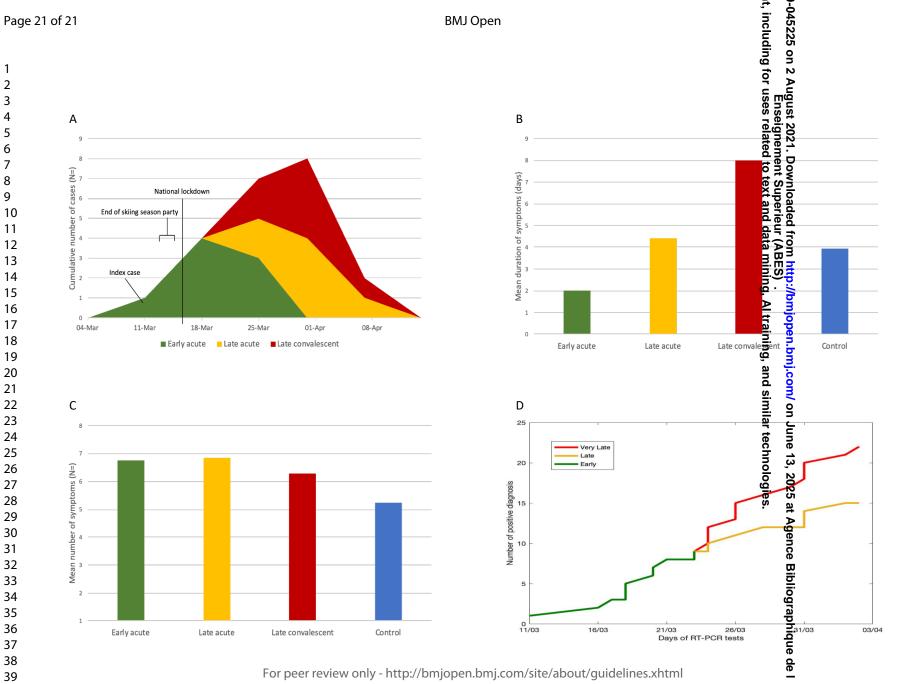
6 589 mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD

 $\frac{7}{2}$  590 (N=1, each). \*\*\*No concordant negatives.

 Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive) in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B) Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms. 

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		STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cofficient studies 도 있	
Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction		ate i 121	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods		and a serie of the	
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure to be and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe fiethods of follow-up	5
		(b) For matched studies, give matching criteria and number of exposed and unexposed 🛓	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifies. Get diagnostic criteria, if	7,8
Data sources/	8*	For each variable of interest, give sources of data and details of methods of assessment (meagurement). Describe	6,7
measurement		comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	6,13
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which are bound by why	7,8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	7,8
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	8
		(e) Describe any sensitivity analyses	NA
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, exagining for eligibility, confirmed	8
		eligible, included in the study, completing follow-up, and analysed       Image: Completing follow-up, and analysed         (b) Give reasons for non-participation at each stage       Image: Completing follow-up, and analysed	8
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information for the posures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	( <i>a</i> ) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their pre to be a structure of the stru	9,10
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaning full period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations		ning b	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of malyses, results from similar studies, and other relevant evidence	12,13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information		ar t	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in controls in case-control studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published exam bles of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine 🛱 rg/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.spide-statement.org.

### Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (REAP-1)

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<b>Primary Subject Heading</b> :	General practice / Family practice

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34 35	48	
36 37	49	ABSTRACT
38	50	<b>Objectives:</b> We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 40	51	regional COVID-19 outbreak in Austria.
41 42	52	Design: Prospective cohort study.
43 44	53	Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.
45 46	54	Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 47	55	April, 2020.
48 49	56	Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
50 51	57	chain reaction (RT-qPCR).
52	58	Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the
53 54	59	outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
55	60	with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
56	61	convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
57 58	62	accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.
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63 Results: Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent), 64 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all 65 acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of 66 symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days 67 (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
 surveillance of coronavirus.

- 74 Strengths and limitations of this study
  - Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
  - Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people testing RT-qPCR reactive were notified within 24 hours.
  - Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
  - The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
  - Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

#### 84 INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.<sup>1</sup> In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,<sup>2</sup> infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that, and gradually declining over 7-10 days.<sup>3-6</sup>

95 SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a
96 large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may
97 cause up to 80% of secondary transmissions.<sup>7</sup> Although symptomatic infection is common (17 %, range 4-41%),
98 the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.<sup>8-10</sup>
99 Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all
100 documented infections.<sup>11</sup> Superspreading events have been reported from across the globe, and countries

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achieving early viral suppression took rapid and decisive action to implement comprehensive case identification and testing, combined with contact tracing and isolation.<sup>12,13</sup> For epidemic control of COVID-19, the effective reproduction number,  $R_e$ , needs to be less than 1; the presence of undetected and persistent infection within the population, even if very small, can increase  $R_e$  and induce a secondary peak of infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward transmission and

106 controlling the virus to protect the public.<sup>14</sup>

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.<sup>15</sup> Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.<sup>15,16</sup> Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and via mobile units.<sup>17</sup> The first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512 cases and 7.389 COVID-19 related deaths have been reported. 

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General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.<sup>18-20</sup> The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."<sup>21</sup> However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.<sup>22,23</sup> In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.<sup>24</sup> The new service supplemented the existing national health hotline for people at risk of COVID-19.25 RT-qPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.<sup>26</sup> Early detection of SARS-CoV-2 is essential for effective contact tracing,<sup>27</sup> and whole genome sequencing may provide data on dynamics of transmission.<sup>28</sup> 

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The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak. 

2 3	139	
4 5	140	METHODS
6 7	141	Setting
8 9	142	This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort
10 11	143	of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was
12	144	conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by
13	145	RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation
14 15	146	of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients
16	147	presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria,
17 18	148	people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the
19	149	hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and
20 21	150	home self-isolate and self-care. Asymptomatic people were excluded from this study.
22 23	151	
24 25	152	Design
26	153	We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2
27 28	154	RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and
29	155	seropositivity data were collected to compare two groups within this cohort of patients:
30		scropositivity data were conceted to compare two groups within any conort of patients.
31 32	156	• Patients testing RT-qPCR reactive at presentation with acute disease
33	157	• Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).
34 35	158	We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR
36	159	and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6
37 38	160	weeks after the acute illness, irrespective of the RT-qPCR result.
39	161	
40		
41 42	162	Intervention
43	163	On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza
44 45	164	Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.
46	165	Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day
47 48	166	appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those
49	167	patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a
50 51	168	minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the
52	169	local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat
53	170	RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2
54 55	171	infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number
56	172	of days from the first patient to the last patient testing RT-qPCR reactive at the GP.
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59 60	173	

Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year, participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country. 

#### **Clinical data**

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK. 

#### 

#### Testing

#### RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).<sup>26</sup> RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection. 

Enzyme linked immune assays (ELISA) 

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),<sup>29</sup> and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.<sup>30</sup> Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of SARS-CoV-2. Reagent wells of the EDI<sup>TM</sup> Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA, 

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212 IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal
213 sample/cutoff (COI).
214 Neutralising antibody assay

Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers  $\geq 10$  were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.<sup>31</sup>

#### 21 223 Outcome measures and statistical analysis

23 224 We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following
 24 25 four testing, viral and genomic outcomes:

Outcome A: The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative. To determine the accuracy of RT-qPCR, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive). 

**Outcome B:** The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters). We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) vs. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent). 

Outcome C: The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent sero-positivity (confirmed infection) to determine any potential correlation between these stages of disease. We used multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody 

positive) and all patients with confirmed infection by calculating the correlation coefficient *r*, and estimating the
95% CI.

Outcome D: The number of viral clades implicated in the outbreak. To do this, SARS-CoV-2 full genome
sequencing was undertaken as part of a wider study covering the whole of Austria.<sup>28</sup> The full-length sequences
were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on
Sharing All Influenza Data (GISAID) database (http://gisaid.org).<sup>32</sup> Sequences were aligned in MEGA7 and nonsynonymous nucleotide variants were identified to determine the respective clades, following the GISAID
classification scheme for lineages.<sup>33</sup>

#### **RESULTS**

#### **Overall testing results**

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

275 Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)
Sex		
Female	14 (63.6%)	9 (60%)
Male	8 (36.4%)	6 (40%)
Age (years)		
16-24	4 (26.7%)	3 (20%)

25-34	4 (26.7%)	2 (13.3%(
35-49	6 (40%)	4 (26.7%)
>50	8 (36.4%)	6 (40%)
Ethnic origin		
White	22 (100%)	15 (100%)

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#### 277 Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

296 Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

303 Regression analysis on confirmed infection
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304 Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of
 305 taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03;

p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible
 covariates of confirmed infection.

	People w (seropositive (N=22)	vith confirmed e, any RT-qP		People with acute disease (RT-qPCR reactive and seropositive) (N=15)				
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value		
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029		
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018		
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039		
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032		
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049		
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607		
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825		
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069		

#### 308 Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

309 Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive,
 310 irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody
 311 confirmed positive) among 15 patients respectively.

## 3839 313 Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

**321** 52

53 322 Correlation between acute and confirmed infection
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Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI
 324 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber
 in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did
 not detect any case with convalescent infection (red curve on Figure 2D).

2 3	327										
4 5	328	Viral clade a	analysis								
6 7	329	Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and tw									· and two
8	330	sequences were not available at the time of analysis. Lineages of SARS-CoV-2 have been identified based on									
9											
10 11	331		mutations in key amino acid positions. <sup>33</sup> Clade G is defined by the mutations D614G, C241T, C3037T and								
12	332	A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N;									
13	333	clade L is mo	clade L is most closely related to the Wuhan reference strain (NC_045512.2). <sup>34</sup> Accordingly, among the 13 viral								
14 15	334	isolates, three	e different clades were i	dentified, ir	ncluding cla	ade L (N=2	), GR (	N=4) and	1 L (N='	7).	
16	335	Table 3: Ger	nomic sequences acces	sed via GIS	SAID listir	ng key ami	no aci	d locatio	ns used	for SAR	S-CoV-2
17 18	336	classificatior	ı.								
19 20		Disease Classification	Virus Name (GISAID)	EPI_ISL_#	Date of RT-qPCR	Lineage	<u>ORF</u> <u>8: 84</u>	<u>ORF3a:</u> <u>57</u>	S:614*	N:203**	N:204**
20		Early acute	hCoV- 19/Austria/CeMM0191/2020	438032	13/03/2020	B(L)	L	Q	D	R	G
22		Early acute	hCoV- 19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
23 24		Early acute	hCoV- 19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	К	R
24		Early acute	hCoV- 19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
26		Early acute	hCoV-	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
27 28		Early acute	19/Austria/CeMM0235/2020 hCoV-	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
29		Early acute	19/Austria/CeMM0250/2020 hCoV-	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
30 31		Early acute	19/Austria/CeMM0222/2020 hCoV-	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
32		Late acute	19/Austria/CeMM0249/2020 hCoV-	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
33		Late acute	19/Austria/CeMM0267/2020 hCoV-	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
34 35		Late acute	19/Austria/CeMM0276/2020 hCoV-	475778	29/03/2020	B.1.8 (G)	L	0	G	R	G
36		Late acute	19/Austria/CeMM0303/2020 hCoV-	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
37			19/Austria/CeMM0324/2020								
38 39		Late acute	hCoV- 19/Austria/CeMM0337/2020	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G
40	337	Caption Tab	ble 3: SARS-CoV-2 cla	ides are cla	ssified by	The Globa	l Initia	tive on S	haring A	All Influe	enza Data
41	338	(GISAID) us	ing specific non-synon	ymous muta	ations in th	e viral gen	ome. C	lade G is	s define	d by the	mutations
42 43	339	D614G, C24	1T, C3037T and A2340	3G in the Sp	pike protein	n; and clade	e GR by	y addition	al RG2	03KR mu	tations in
44	340	the Nucleoca	psid protein N; clade L i	s most close	ely related to	o the Wuha	n refere	ence strain	n (NC_0	45512.2)	. <sup>34</sup> Whole
45	341	genome data	were available for 13/15	sequences;	; data for tw	o sequence	es were	not avail	able at tl	he time of	f analysis.
46 47	342	Accordingly,	among the 13 sequence	es analysed,	three diffe	rent clades	were i	dentified,	includi	ng clades	L (N=2),
48	343	GR (N=4) ar	nd G (N=7). All three c	lades were	detected ir	n early acut	te infec	tion, and	l clade (	G was ad	ditionally
49 50	344	· · · ·	ate acute infection. *Fo			2		· · · · ·			2
50 51	345		ing clade G is shown.			•			-	· · · · ·	
52	346	-	e GR are also shown in							ocupsiu j	
53	340 347	acrining cidu		BICY. UKF,	openiteaul	ng name.					
54 347 55											
56 57	348	DISCUSSIO	N								
58	349	Our results d	lemonstrate that SARS-	CoV-2 RT-	-qPCR test	ing, when a	added	to a natic	onal infl	uenza su	rveillance
59 60	350	programme in	n primary care, can rapi	dly, early a	nd accurate	ly diagnose	e COV	D-19 du	ring an c	outbreak.	Of the 73

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains. 

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,<sup>35</sup> we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy. 

Loss of taste of smell has been recognised as an important marker of COVID-19;36.37 however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.<sup>38</sup> Furthermore, loss of taste could not be objectively confirmed in one third of people<sup>38</sup> suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.<sup>39</sup> Timely and accurate testing is also a prerequisite for effective contact tracing.27 

46 380

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or Page 15 of 22

#### **BMJ** Open

did not attend the practice because of mild disease.<sup>40</sup> No further endemic cases were detected after the outbreak. This suggests that combination prevention including rapid testing and case notification in primary care, contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening sites can effectively detect and control a regional outbreak.<sup>21</sup> 

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.<sup>41</sup> However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),<sup>42,43</sup> but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,9 asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.28 

To our knowledge, this is the first study to show that primary care can contribute to early case detection and termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients, public health, and health systems; nationally and internationally for outbreak epidemiology and control. As

countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number  $R_e$  below 1. Austria has increased the number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.<sup>44</sup> Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2

439 lateral flow antigen testing.

#### 441 CONCLUSIONS

442 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with 443 mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of 444 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 445 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national 446 sentinel surveillance of coronavirus.

448 Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study.
449 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
450 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
451 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
452 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
453 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
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462 Ethics and consent: The Medical University of Graz Research Ethics Committee (reference number: 32-429 ex
 463 19/20) approved collection of anonymised RT-PCR and antibody status data, and the Medical University of
 464 Vienna Research Ethics Committee (reference number: EK1339/2017) additionally approved usage of
 465 anonymised RT-PCR data collected as part of the National Influenza Surveillance Network including generation

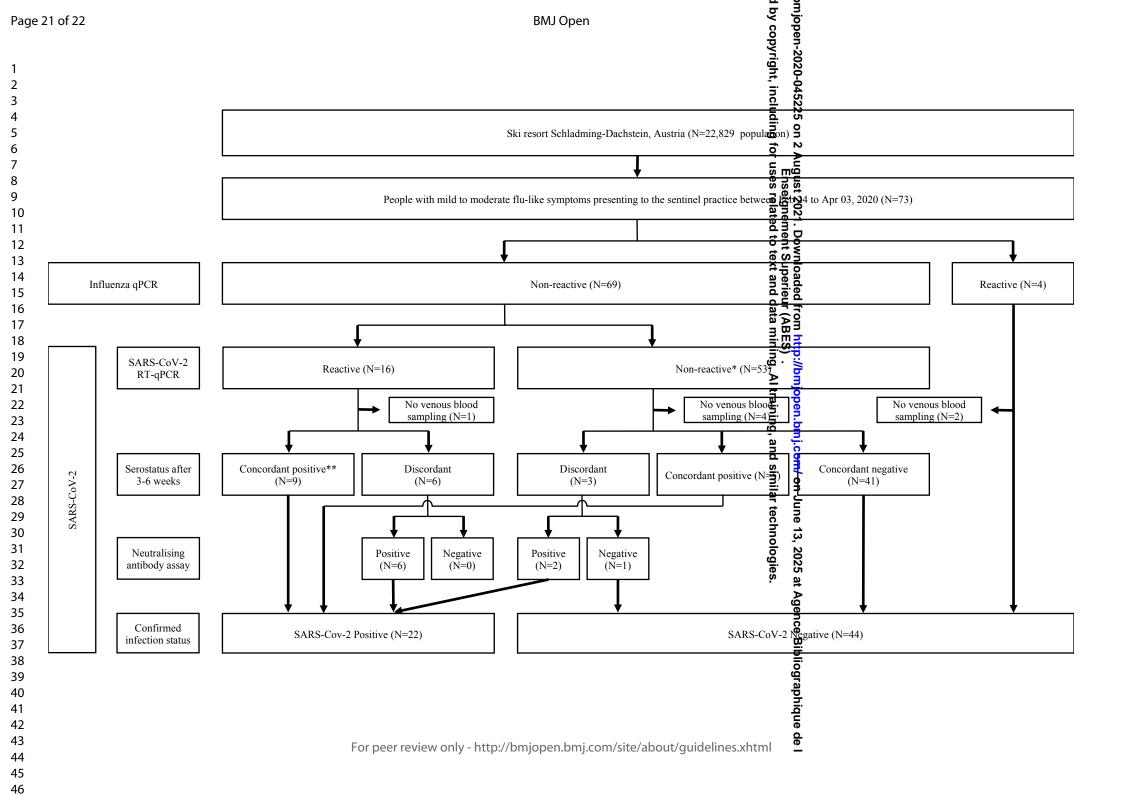
	dary genomic data. Written consent was obtained from all participating patients agreeing on ised data collection for data validation, quality control and research purposes.
Patient	and public involvement: No patient involvement.
Data av	ailability statement: The datasets used and/or analysed during the current study are available from the
correspo	onding author on reasonable request.
Compe	ting Interests: None declared.
Referen	ices
	World Health Organization (WHO). Clinical management of severe acute respiratory infection OVID-19 is suspected, 2020. <u>https://www.who.int/publications-detail/clinical-management-of-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected</u> (Accessed , 2021).
2. From P 577-82.	Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) ublicly Reported Confirmed Cases: Estimation and Application. <i>Ann Intern Med</i> 2020; <b>172</b> (9):
3. COVID-	Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with 2019. <i>Nature</i> 2020; <b>581</b> (7809): 465-9.
	Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-CoV ad dynamics, duration of viral shedding and infectiousness: a living systematic review and meta- s. <i>medRxiv</i> 2020: 2020.07.25.20162107.
	Byrne AW, McEvoy D, Collins AB, et al. Inferred duration of infectious period of SARS-CoV-2: coping review and analysis of available evidence for asymptomatic and symptomatic COVID-19 <i>BMJ Open</i> 2020; <b>10</b> (8): e039856.
6. <i>Infect L</i>	Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. <i>Clin</i> Dis 2020.
7. using o	Endo A, Abbott S, Kucharski AJ, Funk S. Estimating the overdispersion in COVID-19 transmission utbreak sizes outside China. <i>Wellcome Open Res</i> 2020; <b>5</b> : 67.
8. sympto	Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus matic COVID-19. <i>Lancet</i> 2021; <b>397</b> (10269): 93-4.
	Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of omatic COVID-19 and its potential for community transmission: Systematic review and meta- s. <i>Official Journal of the Association of Medical Microbiology and Infectious Disease Canada</i> 2020; 23-34.
10. their clo	Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of ose contacts in Shenzhen, China: a retrospective cohort study. <i>Lancet Infect Dis</i> 2020.
11. novel co	Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of pronavirus (SARS-CoV-2). <i>Science</i> 2020; <b>368</b> (6490): 489-93.
https://	European Centre for Disease Prevention and Control (ECDC). Rapid Risk Assessment: virus disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update, 2020. <u>/www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-</u> 9-pandemic-ninth-update (accessed May 05, 2020).
13. a mode	Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: lling study. <i>Lancet Infect Dis</i> 2020.

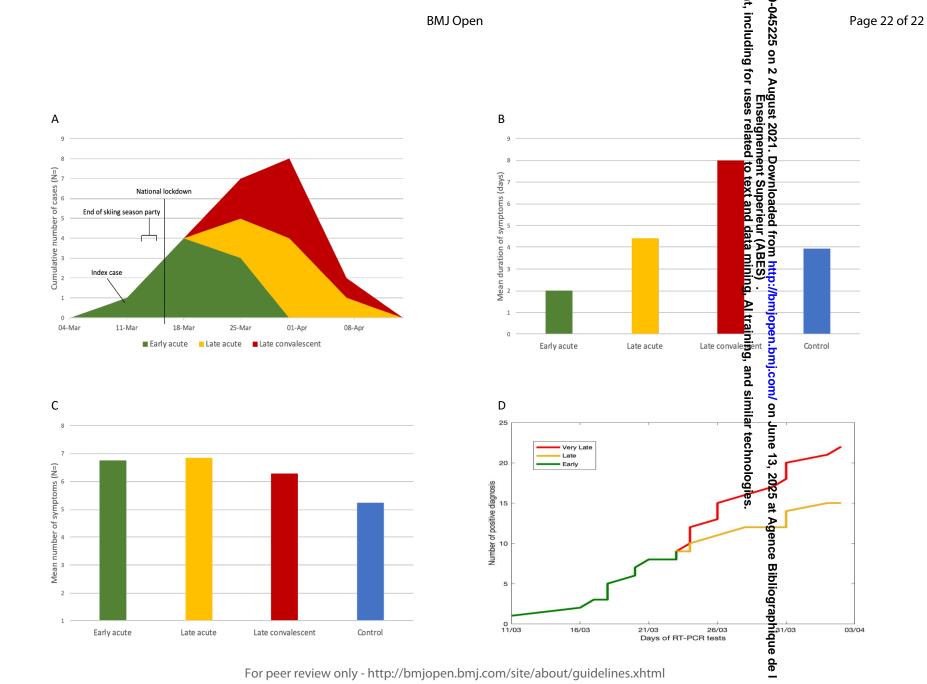
14. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis 2020; 26(6): 1059-66. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in 15. Austria. Wien Klin Wochenschr 2020: 1-8. Popa A, Genger J-W, Nicholson M, et al. Mutational dynamics and transmission properties of 16. SARS-CoV-2 superspreading events in Austria. *bioRxiv* 2020: 2020.07.15.204339. The Independent. 2020. https://www.independent.co.uk/news/world/europe/coronavirus-17. austria-cases-covid-19-hospital-lockdown-latest-a9466281.html (accessed May 05, 2020). 18. European Centre for Disease Prevention and Control (ECDC). Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK –ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessmentcoronavirus-disease-2019-ninth-update-23-april-2020.pdf (accessed June 02, 2020). de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. 19. Eur | Gen Pract 2020; 26(1): 58-60. 20. Hull SA, Williams C, Ashworth M, Carvalho C, Boomla K. Suspected COVID-19 in primary care: how GP records contribute to understanding differences in prevalence by ethnicity. *medRxiv* 2020: 2020.05.23.20101741. European Centre for Disease Prevention and Control (ECDC). Strategies for the surveillance of 21. COVID-19, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020). 22. Roehr B. Covid-19 is threatening the survival of US primary care. *BMJ* 2020; **369**: m2333. Harding-Edgar L, McCartney M, Pollock AM. Test and trace strategy has overlooked importance 23. of clinical input, clinical oversight and integration. *Journal of the Royal Society of Medicine* 2020; (11): 428-32. 24. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (accessed May 28, 2020). 25. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020. 26. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR, 2020. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c\_2 (accessed July 02, 2020). 27. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. The Lancet Public Health. 28. Popa A, Genger JW, Nicholson MD, et al. Genomic epidemiology of superspreading events in Austria reveals mutational dynamics and transmission properties of SARS-CoV-2. Sci Transl Med 2020; 12(573). 29. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol* 2020; **57**(1): e100. 30. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. *J Korean Med Sci* 2020; **35**(14): e149. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation 31. studies of complex interventions (StaRI): a systematic review and e-Delphi. Implementation Science 2015; 10(1): 42. 

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3 4 5 6	558 559 560	32. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. <i>Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin</i> 2017; <b>22</b> (13).
7 8	561 562	33. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. <i>Frontiers in Microbiology</i> 2020; <b>11</b> (1800).
9 10 11 12	563 564 565 566	34. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020.
13 14 15 16	567 568 569	35. Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. <i>The Lancet Child &amp; Adolescent Health</i> .
17 18	570 571	36. Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The Association of "Loss of Smell" to COVID-19: A Systematic Review and Meta-Nnalysis. <i>Am J Med Sci</i> 2020.
19 20 21 22	572 573 574	37. von Bartheld CS, Hagen MM, Butowt R. Prevalence of Chemosensory Dysfunction in COVID-19 Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. <i>ACS Chem</i> <i>Neurosci</i> 2020; <b>11</b> (19): 2944-61.
23 24	575 576	38. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in 2013 European Patients With Mild to Moderate COVID-19. <i>Ann Intern Med</i> 2020.
25 26	577 578	39. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. <i>Nat Med</i> 2020.
27 28 29	579 580	40. Maltezou HC, Vorou R, Papadima K, et al. Transmission dynamics of SARS-CoV-2 within families with children in Greece: A study of 23 clusters. <i>J Med Virol</i> 2020.
30 31	581 582	41. Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper Airway Symptoms. <i>Ear Nose Throat J</i> 2020: 145561320920762.
32 33 34	583 584	42. Sung Y-T, Wu J-S. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-RRP): A new technique for psychological measurement. <i>Behav Res Methods</i> 2018; <b>50</b> (4): 1694-715.
35 36	585 586	43. Rojas-Lechuga MJ, Izquierdo-Domínguez A, Chiesa-Estomba C, et al. Chemosensory dysfunction in COVID-19 out-patients. <i>Eur Arch Otorhinolaryngol</i> 2020: 1-8.
37 38 39 40	587 588 589	44. NHS England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020. 2020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-2020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed May 05, 2021).
41	590	
42 43 44	591	FIGURE LEGENDS
44 45	592	Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15
46	593	patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-
47 48	594	qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing
49	595	antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant
50 51	596	antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44
52	597	patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1
53	598	patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with
54 55 56	599	Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious
	600	mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD
57 58	601	(N=1, each). ***No concordant negatives.
59 60	602	

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3 4	603	Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main
5	604	outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing
6 7	605	season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last
8	606	endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)
9 10	607	in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute
11	608	infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B)
12 12	609	Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive
13 14	610	among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters;
15	611	(C) Mean duration of symptoms; and (D): Mean number of symptoms.
16 17	612	
18 19	613	
20	614	among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms.
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		뜻 冷 STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of <i>coffort studies</i> 은 있	
Section/Topic	ltem #	Recommendation	Reported on page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		لة مع مع (b) Provide in the abstract an informative and balanced summary of what was done and what (المن المعرفة) (b) Provide in the abstract an informative and balanced summary of what was done and what (المن المعرفة) (b) (b) (b) (b) (b) (b) (b) (b) (b) (b	2,3
Introduction	1	ated	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported $6220$	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods	1		
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure the w-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe Bethods of follow-up	5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifies. Get diagnostic criteria, if	7,8
Data sources/	8*	For each variable of interest, give sources of data and details of methods of assessment (meagurement). Describe	6,7
measurement		comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	6,13
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which rot in the analyses of the second s	7,8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7,8
		(b) Describe any methods used to examine subgroups and interactions	7,8
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	8
		(e) Describe any sensitivity analyses	NA

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, exaginined for eligibility, confirmed	8
		eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information for methods and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	( <i>a</i> ) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,10
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaning full approximate period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion		A Bu	
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations		n.b	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of malyses, results from similar studies, and other relevant evidence	12,13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information		arte	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in controls in case-control studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine grg/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.spote-statement.org.

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