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25

26 **Abstract**

27 Wastewater surveillance to monitor the incidence of infections faces challenges, in terms of
28 discrepancies with sentinel-confirmed cases. We examined whether testing rates could explain the
29 discrepancy between SARS-CoV-2 RNA found in a City of Sapporo wastewater treatment plant and
30 the number of infections recorded at Hokkaido University Hospital over a period of approximately
31 four years. Then, we analyzed the association between wastewater RNA concentrations with
32 incidences of new cases among hospital-acquired infections. Linear regression analyses were
33 performed using wastewater RNA concentrations as the independent variable and infected cases with
34 and without correction for the testing rate as the dependent variable. In addition, modified Poisson
35 regression analyses were performed, with the incidence of new cases among hospital-acquired
36 infections as the dependent variable. After the legal reclassification of COVID-19 in Japan was
37 changed to the same category as seasonal influenza, the rate of hospital testing declined significantly,
38 though wastewater RNA concentrations remained high. Compared to non-correction for testing rates,
39 corrected community-acquired infection cases showed a stronger association with wastewater RNA
40 concentrations ($R^2 = 0.54$ and 0.75 , respectively). The incidence of hospital-acquired infections was
41 positively associated with wastewater RNA concentrations (incidence risk rate: 2.24 [95%
42 confidence interval: 1.36–3.71]), and a \log_{10} wastewater RNA concentration [copies/L] of 4.57
43 (4.10–5.03) was suggested as a 25% probability of new incidence. This study emphasized that
44 SARS-CoV-2 wastewater surveillance is an objective and useful indicator reflecting infection

45 incidence independent of testing rates.

46

47 **Keywords**

48 Community infection; COVID-19; infection control; nosocomial infection; wastewater-based

49 epidemiological monitoring; wastewater-based epidemiology

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51 **1. Introduction**

52 More than five years after the onset of the coronavirus disease 2019 (COVID-19) pandemic,

53 individuals with underlying conditions such as cardiovascular disease, diabetes, chronic kidney

54 disease, and malignancies, as well as older adults, remain at high risk of severe outcomes

55 (Dryden-Peterson et al., 2025). With this in mind, it is important to monitor COVID-19 trends in

56 communities, long-term care facilities, and hospitals.

57 Beginning February 1, 2020, COVID-19 was managed under Japan’s Infectious Diseases Control

58 Law as a “designated infectious disease” (operationally, Category □–equivalent), and from February

59 13, 2021 onward, it was reclassified as a “pandemic influenza” infectious disease (also Category

60 □–equivalent), a status that remained until May 7, 2023. On May 8, 2023, Japan reclassified

61 COVID-19 as a Category □ disease, aligning it with seasonal influenza. As a result, national case

62 surveillance shifted from universal (all-case) reporting to sentinel surveillance (Sawakami et al.,

63 2021). The costs of clinical testing and therapy have also become chargeable to patients.

64 Beginning in March 2020, several countries, including the Netherlands, the United States, Australia,
65 and France, implemented wastewater surveillance for severe acute respiratory syndrome coronavirus
66 2 (SARS-CoV-2) to complement the limitations of sentinel surveillance, such as incomplete coverage
67 of the population, potential reporting delays, and dependence on healthcare-seeking behaviors (Yang
68 et al., 2023). In Japan, multiple municipalities have adopted a dual surveillance system of wastewater
69 and sentinel surveillance since 2020, using wastewater data to complement sentinel data in assessing
70 epidemic dynamics (Japan Institute for Health Security, 2025; Murakami et al., 2024).

71 In the City of Sapporo, Japan, wastewater surveillance for SARS-CoV-2 has been conducted since
72 February 2021 (City of Sapporo, 2025). During the pandemic, SARS-CoV-2 RNA concentrations in
73 wastewater closely tracked with confirmed cases in the city (Ando et al., 2023; Murakami et al.,
74 2024) and at the hospitals (Kagami et al., 2023; Kagami et al., 2025). However, following the
75 disease's shift to endemic status, on May 8, 2023, a discrepancy between sentinel-confirmed cases
76 and wastewater RNA levels progressed over time (Figure S1), as demonstrated previously (Kagami
77 et al., 2025; Murakami et al., 2024). Such discrepancies have been documented in other settings that
78 integrate wastewater surveillance with case-based sentinel surveillance, including in the United
79 Kingdom, Denmark, Singapore, South Africa, Portugal, and Argentina (Iwu-Jaja et al., 2023; Jones et
80 al., 2025; Krogsgaard et al., 2024; Masachessi et al., 2022; Monteiro et al., 2022; Wong et al., 2023).

81 Potential contributors to these discrepancies include: (1) increased proportions of asymptomatic or

82 mild infections (Nakakubo et al., 2023), (2) reduced clinical testing (Boehm et al., 2023), (3) changes
83 in healthcare-seeking behaviors (Maree et al., 2025), (4) variations in viral shedding patterns by age
84 (Omori et al., 2021), and (5) differences in fecal excretion or environmental stability associated with
85 emerging variants (Prasek et al., 2023; Sherchan et al., 2023). This study aimed to assess whether
86 adjusting for the testing rate, corresponding to point (2) above, reduced clinical testing (Boehm et al.,
87 2023), improves the association between wastewater RNA levels and confirmed cases during the
88 endemic period. A previous study (Boehm et al., 2023) illustrated this issue by showing that, between
89 late 2021 and early 2022, the widespread adoption of at-home antigen test kits, combined with the
90 fact that positive results from these tests were not reported to public health authorities, led to a
91 discrepancy between SARS-CoV-2 RNA concentrations in wastewater and the number of newly
92 reported COVID-19 cases. The primary objective of this study was to investigate the discrepancy
93 between wastewater surveillance and COVID-19 cases using a relatively small-scale but highly
94 accurate dataset from a university hospital, comprising COVID-19 cases and the number of
95 SARS-CoV-2 tests performed. We analyzed wastewater RNA concentration data from the City of
96 Sapporo alongside COVID-19 cases and SARS-CoV-2 testing data from Hokkaido University
97 Hospital. Furthermore, as a secondary objective, building on our previous finding of an association
98 between wastewater SARS-CoV-2 RNA concentrations and the number of hospital-acquired
99 COVID-19 cases among inpatients (Kagami et al., 2025), we examined whether wastewater
100 SARS-CoV-2 RNA concentrations can be used to signal the incidence risk of hospital-acquired

101 COVID-19 infections. A previous study (Kagami et al., 2025) reported weaker correlations between
102 viral concentrations in wastewater with cases of hospital-acquired infections than with
103 community-acquired cases. In this study, we focused on explaining the probability of new incidences
104 using the viral concentrations in wastewater.

105

106 **2. Materials and Methods**

107 **2.1. Ethical approval**

108 Ethical approval for this study was obtained from the Institutional Review Board of Hokkaido
109 University Hospital for Clinical Research (025-0012).

110

111 **2.2. SARS-CoV-2 RNA analysis from wastewater samples**

112 SARS-CoV-2 RNA concentrations in wastewater were obtained from a wastewater surveillance
113 program conducted by the City of Sapporo. Other studies have also used data obtained from this
114 program (Kagami et al., 2023; Kagami et al., 2025; Murakami et al., 2024). In this study, 24-hour
115 composite untreated wastewater samples collected from Week 7 of 2021 (February 15–21, 2021)
116 through Week 13 of 2025 (March 24–30, 2025), excluding Weeks 9–18 of 2023 and Week 1 of 2025,
117 were used. Samples were collected from five catchment areas covered by three wastewater treatment
118 plants in the City of Sapporo (each catchment area covers approximately 164,700 to 246,300 people,

119 accounting for 52% of the city’s population of approximately 1.96 million), except from Weeks 7–14
120 of 2021, when samples were collected from three catchment areas. Sampling was generally
121 conducted three times per week at each catchment area from Week 7 of 2021 to Week 39 of 2023,
122 and once per week thereafter. Therefore, 9, 15, and 5 samples were collected per week from Weeks
123 7–14 of 2021, from Week 15 of 2021 through Week 39 of 2023, and from Week 40 of 2023 through
124 Week 13 of 2025, respectively. SARS-CoV-2 RNA concentrations in wastewater were measured
125 using the EPISENS-S (Efficient and Practical virus Identification System with ENhanced Sensitivity
126 for Solids) method (Ando et al., 2022), with a theoretical limit of detection (LOD) of 93 copies/L.
127 Briefly, solids in each wastewater sample were recovered via low-speed centrifugation, followed by
128 direct RNA extraction from the pellet, multiplex one-step RT-preamplification, and quantitative PCR.
129 Details of the EPISENS-S method were described in a previous study (Ando et al., 2022). To
130 eliminate the effects of dilution by rainwater, the obtained wastewater concentrations were corrected
131 using values adjusted for flow rate to the wastewater treatment plant. This same correction was used
132 in a previous study (Kagami et al., 2025).

133

134 **2.3. Monitoring confirmed COVID-19 cases and hospital testing rates**

135 Data on individuals infected with COVID-19—including age, sex, case category (outpatient, hospital
136 staff member, those infected outside the hospital but developed symptoms after admission, or
137 hospital-acquired infection), and date of case ascertainment— and the number of tests performed

138 each week were extracted from reports by the Department of Infection Control and Prevention at
139 Hokkaido University Hospital. The categories of outpatient, hospital staff member, and those
140 infected outside the hospital but developed symptoms after admission were consolidated into a single
141 category, community-acquired infections. Testing data were then classified into one of two groups,
142 community- or hospital-acquired infection. Testing for community-acquired infections among
143 hospital staff members was partially conducted prior to COVID-19's reclassification, but not after its
144 reclassification. Therefore, as a sensitivity analysis, we also analyzed community-acquired infections
145 excluding staff members.

146

147 **2.4. Statistical analysis**

148 For SARS-CoV-2 RNA concentrations in wastewater and the cases of community-acquired
149 infections, total infections, and community-acquired infections excluding staff members, we
150 examined how non-detected data were replaced and whether these data were close to normal or
151 log-normal distribution. Among the 2,213 data points of SARS-CoV-2 concentration in wastewater,
152 249 were below the LOD (93 copies/L). Among the 204-weekly data of community-acquired
153 infection, 28 were below the minimum reported case (MRC; 1 person/week). Similarly, 27 were
154 below the MRC (1 person/week) among the 204 data of total infection cases. The
155 community-acquired infections excluding staff members showed < 1 person/week in 51 out of 204
156 data. Data replacement for values below the LOD or MRC was performed in accordance with the

157 protocols of a previous study (Murakami et al., 2024) using the value corresponding to half of the
158 non-detected rate based the distribution estimation for left-censored data. This was conducted for
159 both normal and log-normal distributions. For SARS-CoV-2 RNA concentrations in wastewater,
160 community-acquired infection cases, total infection cases, and community-acquired infection cases
161 excluding staff members, we assumed a normal distribution resulted in negative values, and that a
162 log-normal distribution would result in values slightly higher than the LOD or MRC, except for
163 community-acquired infection cases excluding staff members (Table S1). Therefore, as Q-Q plots are
164 effective for evaluating normality when a sample size is large (Mishra et al., 2019), these were
165 created using the following replacements: 0 for a normal distribution and the LOD or MRC value for
166 a log-normal distribution, except community-acquired infection cases excluding staff members.
167 Regarding community-acquired infection cases excluding staff members, the value corresponding to
168 half of the non-detected rate was used for the for a log-normal distribution. All four
169 variables—SARS-CoV-2 RNA concentration in wastewater, community-acquired infection cases,
170 total infection cases, and community-acquired infection cases excluding staff members—were closer
171 to a log-normal distribution than to a normal distribution (Figure S2). Therefore, in the following
172 analysis, except for community-acquired infection cases excluding staff members, non-detected data
173 were replaced with the LOD or MRC, and \log_{10} values were used for SARS-CoV-2 RNA
174 concentrations in wastewater, community-acquired infection cases, and total infection cases.
175 Regarding community-acquired infection cases excluding staff members, non-detected data were

176 replaced with the value corresponding to half of the non-detected rate, and \log_{10} values were used.

177 Next, linear regression analyses were performed with the \log_{10} values of community-acquired
178 infection and total infection cases as the dependent variables and the \log_{10} values of SARS-CoV-2
179 RNA concentrations in wastewater as the independent variable. The representative value of
180 SARS-CoV-2 RNA concentrations in wastewater was calculated as the geometric mean of the
181 measured values obtained during one week, following the protocols used in a previous study
182 (Murakami et al., 2024). A positive correlation was observed between COVID-19 cases and
183 SARS-CoV-2 RNA concentrations in wastewater taken from the same target site between February
184 2021 and February 2023, with the highest correlation observed when the lag time was set to 0 weeks
185 (Kagami et al., 2023). Therefore, we used the values of wastewater concentration in the same week
186 as the community-acquired infection and total infection cases. To evaluate the impact of changes of
187 in testing rates on the discrepancy between wastewater SARS-CoV-2 RNA concentrations and
188 confirmed cases after the disease's reclassification to Category \square , we divided the
189 community-acquired infection and total infection cases by the ratio of the number of clinical tests
190 performed in the corresponding week to determine the average number of clinical tests per week
191 before the reclassification, and then used its \log_{10} value as the dependent variables. The testing rate
192 for community-acquired infections was used for those cases, while the total number of tests
193 administered was used for total infection numbers. As described above, the testing rate for
194 community-acquired infections among hospital staff members was not fully ascertained. In this

195 analysis, we assumed that the trend of testing within the hospital represented that of the region as a
196 whole. To complement the assumption, as a sensitive analysis, linear regression analyses were
197 performed with the \log_{10} values of community-acquired infections, excluding staff members, as the
198 dependent variables and the \log_{10} value of SARS-CoV-2 RNA concentrations in wastewater as the
199 independent variable. For the correction, the clinical testing rate for community-acquired infections
200 excluding staff members was used instead.

201 Furthermore, we analyzed whether the new cases among hospital-acquired infections could be
202 explained using SARS-CoV-2 RNA wastewater concentration data collected the same week. We
203 defined weeks without incidence among hospital-acquired infections as “0,” weeks with new
204 infections (i.e., the week when a new hospital-acquired infection was acquired) as “1,” and excluded
205 weeks with occurrences among hospital-acquired infections in both the previous and corresponding
206 weeks. A total of 137 weeks were included, of which 22 weeks (16%) had a new case of
207 hospital-acquired infection. We performed a modified Poisson regression analysis (i.e., Poisson
208 regression analysis with a robust error variance) with the new incidence among hospital-acquired
209 infections as the dependent variable and the \log_{10} value of the SARS-CoV-2 RNA concentration in
210 wastewater as the independent variable. The modified Poisson regression analysis was chosen
211 because, even when the prevalence was 10% or higher, the incidence rate ratio it calculated was close
212 to the relative risk (McNutt et al., 2003; Zou, 2004). Based on the partial regression coefficients
213 obtained, we calculated the \log_{10} value of SARS-CoV-2 RNA concentration in wastewater (95%

214 confidence interval [CI]) corresponding to a 25% probability of a new incidence. The value of 25%
215 was chosen because it is slightly higher than the 16% prevalence of the new incidence rate observed
216 throughout the survey period in this study, making it an appropriate threshold for alert.

217 Finally, we analyzed the association of the \log_{10} value of the SARS-CoV-2 RNA concentration in
218 wastewater at the start of the new incidence with the number of continuous weeks from the start
219 through the end of new incidence among hospital-acquired infections (hereinafter referred to as an
220 “event”) and the total number of hospital-acquired infections during the event. After using Q-Q plots
221 to confirm that both the number of continuous weeks and the total number of hospital-acquired
222 infections per event were closer to a log-normal distribution than to a normal distribution (Figure S3),
223 we performed linear regression analyses with their \log_{10} values as the dependent variables and the
224 \log_{10} values of SARS-CoV-2 RNA concentration in wastewater during the week when the new
225 incidence among hospital-acquired infections appeared as the independent variable.

226 The analysis was performed using R (R Development Core Team, 2025); R packages “NADA” (Lee,
227 2022), “sandwich” (The Comprehensive R Archive Network, 2025b), and “lmtest” (The
228 Comprehensive R Archive Network, 2025a); and IBM SPSS version 28.

229

230 **3. Results**

231 Figure 1(a–c) and Table S2 show the temporal changes in SARS-CoV-2 RNA concentrations in

232 wastewater in the City of Sapporo, the confirmed COVID-19 cases, and the number tests conducted
233 at Hokkaido University Hospital. The number of confirmed COVID-19 cases corrected by the
234 number of tests performed are also shown in Figure 1(d). Table 1 presents the characteristics of
235 community-acquired infections, hospital-acquired infections, total infections, and the ages of those
236 infected before and after COVID-19's reclassification to Category □. In general, SARS-CoV-2 RNA
237 concentrations in wastewater remained high even after the reclassification, but the numbers of
238 community-acquired infections and total infection cases peaked before the reclassification. The
239 number of tests conducted decreased considerably after the reclassification. Significant positive
240 associations were observed between SARS-CoV-2 RNA concentrations in wastewater and the
241 numbers of community-acquired and total infections (Figures 2a–d). Without correcting based on the
242 testing rate, the R^2 values were 0.54 ($P < 0.001$) for community-acquired infections and 0.57 ($P <$
243 0.001) for all infections. Infection cases after the reclassification were lower than those estimated
244 using the regression equations. In contrast, after correcting for the testing rate, the R^2 values
245 improved to 0.75 ($P < 0.001$) for community-acquired infections and 0.78 ($P < 0.001$) for all cases
246 (Figure 2c, d). The slopes with correction using the testing rate were 0.85 (95% CI: 0.78–0.91) for
247 community-acquired infections and 0.66 (95% CI: 0.62–0.71) for all cases, both of which were
248 significantly lower than 1. A similar result was obtained for community-acquired infections
249 excluding staff members (Figure S4). The R^2 value was 0.44 ($P < 0.001$) for non-corrected data
250 based on the testing rate and 0.62 ($P < 0.001$) for corrections accounting for the number of tests

251 conducted. The slope with correction was 0.67 (95% CI: 0.60–0.74).

252 A significant positive association was observed between the SARS-CoV-2 RNA concentration in
253 wastewater and the new incidences of hospital-acquired infections (Figure 3). The incidence risk rate
254 of \log_{10} SARS-CoV-2 RNA concentration based on new incidences among hospital-acquired
255 infections was 2.24 (95% CI: 1.36–3.71) ($P = 0.002$), and the \log_{10} for SARS-CoV-2 RNA
256 concentration [copies/L] at which there was a 25% probability of a new incidence was 4.57 (95% CI:
257 4.10–5.03). The SARS-CoV-2 RNA concentration in wastewater at the onset of a new case showed a
258 weak, but significantly positive, association with the number of continuous weeks and the total
259 number of hospital-acquired infections per event ($R^2 = 0.26$, $P = 0.016$; $R^2 = 0.23$, $P = 0.024$,
260 respectively) (Figure 4).

261

262 **4. Discussion**

263 This study investigated the association between SARS-CoV-2 RNA concentrations in wastewater
264 samples collected in Sapporo, Japan with confirmed cases of COVID-19 at Hokkaido University
265 Hospital over a period of approximately four years. With the disease's reclassification to Category □,
266 the testing rate decreased considerably. After correcting for this, strong associations were
267 demonstrated between SARS-CoV-2 RNA concentrations in wastewater and cases of
268 community-acquired infection or total infections at the hospital. This was also confirmed by a

269 sensitivity analysis that excluded staff members. In the same study site, we found that a strong
270 correlation between SARS-CoV-2 RNA concentrations in wastewater and the confirmed number of
271 COVID-19 cases was reported before the reclassification (Kagami et al., 2023), while the infection
272 cases per SARS-CoV-2 RNA concentration in wastewater was reduced after the reclassification
273 (Kagami et al., 2025). These results are consistent with the findings of previous studies (Kagami et
274 al., 2023; Kagami et al., 2025) and provided new insights suggesting that COVID-19 cases at the
275 hospital could be sufficiently explained by SARS-CoV-2 RNA concentrations in wastewater, after
276 correcting for the testing rate. Therefore, the divergence between SARS-CoV-2 RNA concentrations
277 in wastewater and COVID-19 cases after the reclassification was largely attributable to a decrease in
278 the number tests conducted (Boehm et al., 2023). This highlights that SARS-CoV-2 wastewater
279 surveillance is an objective indicator that reflects infection incidence, including asymptomatic
280 infections, without being influenced by factors such as changes in hospital visiting behaviors and the
281 number of tests conducted.

282 This study has also demonstrated that new cases among hospital-acquired infections were associated
283 with SARS-CoV-2 RNA concentrations in wastewater. Considering that SARS-CoV-2 RNA
284 concentrations in wastewater reflect community infection incidence, this suggests that
285 hospital-acquired infections might occur through visits to hospitals by infected patients, hospital staff
286 members, or members of the community (Hatfield et al., 2023; Kagami et al., 2025). Moreover, the
287 positive associations between SARS-CoV-2 RNA concentrations in wastewater with the number of

288 continuous weeks and the total number of hospital-acquired infections per event suggest that higher
289 concentrations were associated with longer-lasting and large numbers of incidences among
290 hospital-acquired infections. Therefore, wastewater surveillance is expected to serve as a valid
291 indicator regarding new incidences among hospital-acquired infections. This study found that a 25%
292 probability of a new incidence corresponded to a \log_{10} SARS-CoV-2 RNA concentration [copies/L]
293 of 4.57 (95% CI: 4.10–5.03).

294 This study has several limitations. First, it was focused only on the association between
295 SARS-CoV-2 RNA concentrations in wastewater in Sapporo and the confirmed number COVID-19
296 cases at Hokkaido University Hospital, so caution is needed when extending these findings to other
297 regions.

298 Second, while the study demonstrated that COVID-19 cases at the hospital could be explained via
299 wastewater SARS-CoV-2 RNA concentrations after correcting for the testing rate, the reasons for the
300 decline in the number of tests conducted after the disease's reclassification remain unclear. One
301 possible interpretation is that individuals avoided hospital visits, due to the introduction of testing or
302 therapy fees after the reclassification to Category □ (Maree et al., 2025). However, other factors,
303 including an increase in asymptomatic or mild cases due to viral attenuation (Nakakubo et al., 2023),
304 cannot be ruled out.

305 Third, we could not completely ascertain the testing rate for community-acquired infections among
306 staff members within the hospital. Therefore, in this study, a sensitivity analysis was performed by

307 excluding staff members infected with COVID-19. When we did this, we could confirm that
308 consistent results were obtained.

309 Fourth, this study did not adequately consider factors regarding the hospital's capacity for individuals
310 infected with COVID-19 or visits to other hospitals. In particular, regressions between the \log_{10}
311 values of SARS-CoV-2 RNA concentrations in wastewater and the \log_{10} values of
312 community-acquired or total infection cases showed that their slopes were less than 1. In a log–log
313 regression equation, a slope of 1 indicates that the two variables are proportional ($Y = a \times X^b \Leftrightarrow$
314 $\log_{10} Y = b \times \log_{10} X + \log_{10} a$). Therefore, wastewater virus concentrations were not proportional to
315 the infected individual cases at the hospital. This result contrasts with a previous study that showed
316 the slope between the \log_{10} values of SARS-CoV-2 RNA concentrations in wastewater and the \log_{10}
317 values of COVID-19 cases in Sapporo was nearly 1 (Murakami et al., 2024). One possible
318 interpretation is that visits to other hospitals might be a factor; therefore, a comprehensive survey
319 targeting individuals infected with COVID-19 across multiple hospital facilities is a promising area
320 for future research.

321

322 **5. Conclusions**

323 This study analyzed the associations between SARS-CoV-2 RNA concentrations in wastewater and
324 confirmed COVID-19 cases at a university hospital, with and without correction accounting for

325 testing conducted within the hospital, to clarify the cause of the discrepancy between wastewater
326 viral concentrations and confirmed cases after COVID's reclassification to Category \square . By
327 correcting for the number of tests conducted at the hospital, the community-acquired infections and
328 total number of infection cases at the hospital could be explained by the SARS-CoV-2 RNA
329 concentration in wastewater, and the discrepancy was interpreted as being mainly due to a decrease
330 in the testing rate.

331 This study emphasized that SARS-CoV-2 wastewater surveillance is an objective indicator reflecting
332 infection incidence that is independent of individuals' hospital visiting behaviors and testing rates.
333 Furthermore, SARS-CoV-2 RNA concentrations in wastewater were positively associated with the
334 probability and total number of new cases among hospital-acquired infections and their continuous
335 time length. As an indicator for alerting to incidence among hospital-acquired infections, a \log_{10}
336 SARS-CoV-2 RNA concentration [copies/L] of 4.57 (95% CI: 4.10–5.03) in wastewater is suggested
337 as a 25% probability of new incidence.

338

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342

343 **Conflicts of Interest**

344 Michio Murakami reports a relationship with NJS Co., Ltd. that includes: consulting or advisory.

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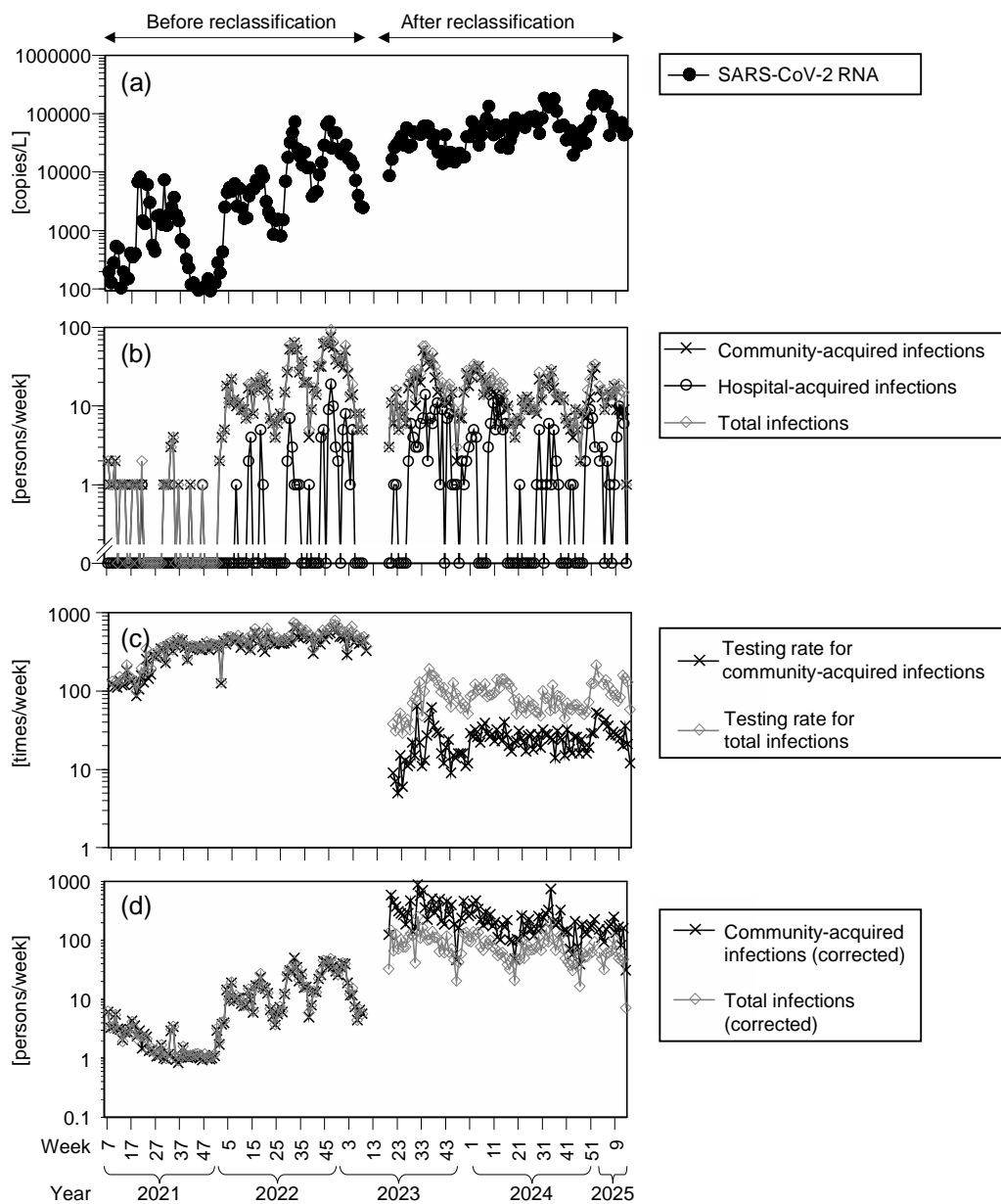
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444 **Figure/Table captions**



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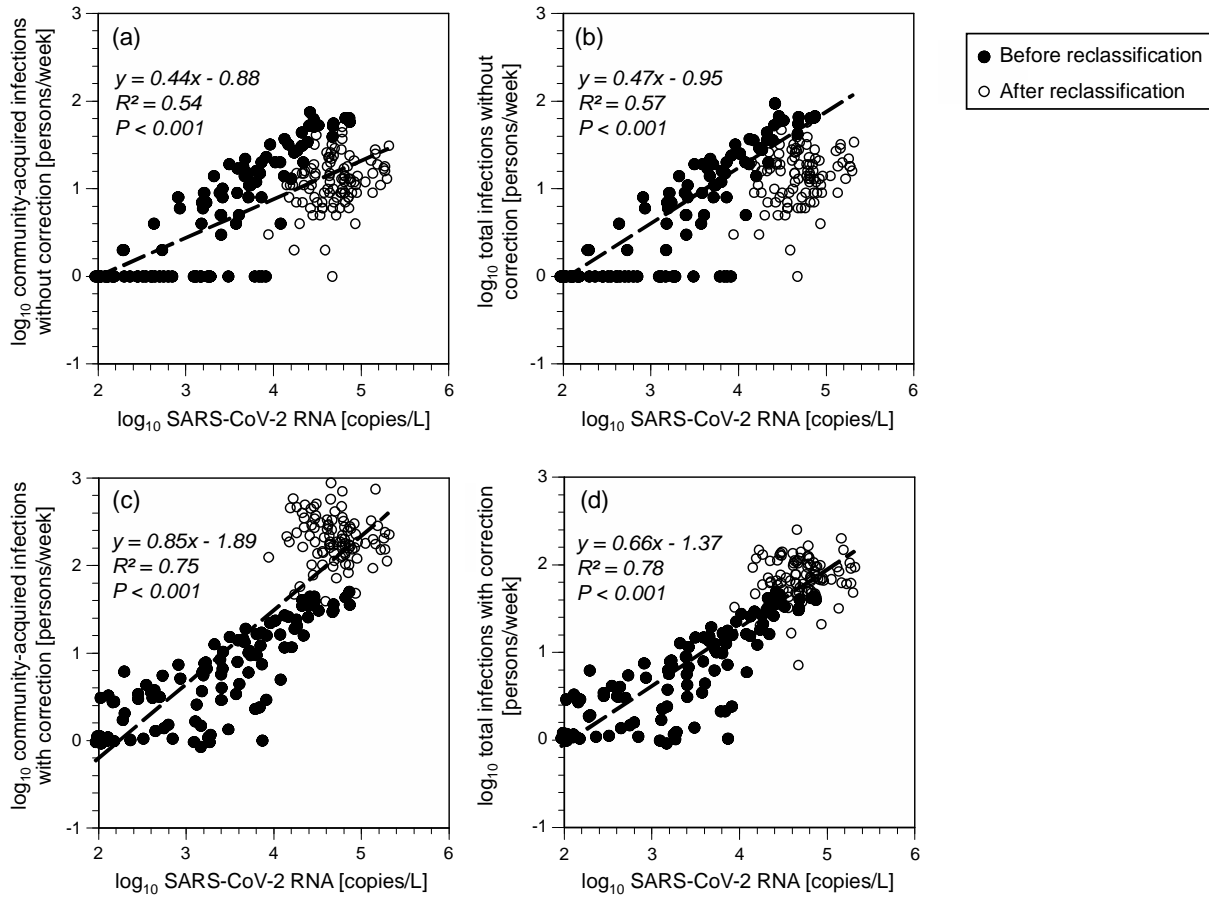
446 Figure 1. Temporal changes in SARS-CoV-2 RNA concentrations in wastewater, confirmed

447 COVID-19 cases, and the testing rate. (a) Wastewater concentrations in the City of Sapporo, (b)

448 Confirmed COVID-19 cases at Hokkaido University Hospital (without correction for testing rate),

449 (c) Hospital testing rate, (d) Confirmed COVID-19 cases at the hospital (with correction for testing

450 rate).



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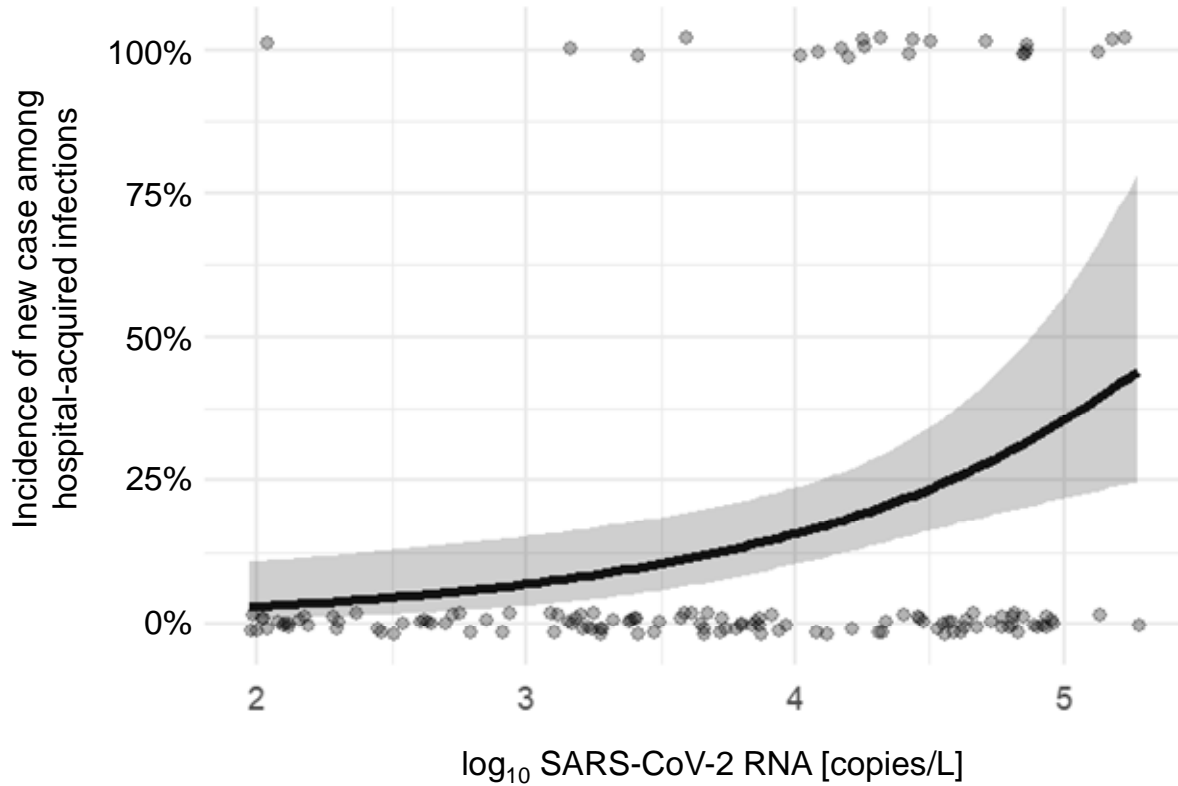
452 Figure 2. Scatterplots of SARS-CoV-2 RNA concentration vs. confirmed COVID-19 cases. (a)

453 Community-acquired infections without correction by the number of testing, (b) Total infections

454 without correction for testing rate, (c) Community-acquired infections with correction for testing rate,

455 (d) Total infections with correction for testing rate.

456



457

458 Figure 3. Associations between SARS-CoV-2 RNA concentrations in wastewater and the incidence
459 of new cases among hospital-acquired infections. Solid line: modified Poisson regression analysis
460 was applied in the prediction. Gray areas represent a 95% confidence interval (CI) of the prediction.

461 Partial regression coefficient (95% CI): intercept, -5.08 (-7.30 — 2.85) ($P < 0.001$); log₁₀

462 SARS-CoV-2 RNA concentrations, 0.81 (0.31 — 1.31) ($P = 0.002$).

463

464

Table 1. Characteristics of individuals infected with COVID-19.

| | | Male | | | Female | | | Total | | |
|-----------------------|-------------------------------|----------|-----------------------|--------------|----------|-----------------------|--------------|----------|-----------------------|--------------|
| | | <i>N</i> | Age (arithmetic mean) | Age (median) | <i>N</i> | Age (arithmetic mean) | Age (median) | <i>N</i> | Age (arithmetic mean) | Age (median) |
| Before classification | Community-acquired infections | 567 | 42 | 38 | 836 | 40 | 37 | 1403 | 41 | 37 |
| | Hospital-acquired infections | 63 | 58 | 66 | 42 | 56 | 62 | 105 | 57 | 62 |
| | Total infections | 630 | 43 | 39 | 878 | 40 | 37 | 1508 | 42 | 38 |
| After classification | Community-acquired infections | 538 | 46 | 41 | 877 | 41 | 38 | 1415 | 43 | 39 |
| | Hospital-acquired infections | 151 | 61 | 66 | 127 | 61 | 66 | 278 | 61 | 66 |
| | Total infections | 689 | 49 | 47 | 1004 | 44 | 40 | 1693 | 46 | 43 |