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Incorporating temporal distribution of population-level viral load enables real-time estimation of COVID-19 transmissibility

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Abstract

Many locations around the world have used real-time estimates of the time-varying effective reproductive number (R_t) of COVID-19 to provide evidence of transmission intensity to inform control strategies. Estimates of R_t are typically based on statistical models applied to case counts and typically suffer lags of more than a week because of the incubation period and reporting delays. Noting that viral loads tend to decline over time since illness onset, analysis of the distribution of viral loads among confirmed cases can provide insights into epidemic trajectory. Here, we analyzed viral load data on confirmed cases during two local epidemics in Hong Kong, identifying a strong correlation between temporal changes in the distribution of viral loads (measured by cycle threshold values) and estimates of R_t based on case counts. We demonstrate that cycle threshold values could be used to improve real-time R_t estimation, enabling more timely tracking of epidemic dynamics.

Introduction

Monitoring the transmissibility of an emerging infectious disease in a timely manner is crucial to monitor the effectiveness of public health and social measures and to inform better control policies. During the coronavirus diseases 2019 (COVID-19) pandemic, real-time assessment of transmissibility has generally been achieved through monitoring the time-varying effective reproductive number, R_t . A number of statistical approaches have been developed to allow estimation of R_t from time series of daily case counts either recorded by date of illness onset or by date of laboratory confirmation¹. However, these approaches tend only to be able to estimate R_t with a lag of one week or more, because COVID-19 transmission can occur prior to illness onset², because of delays between individuals being infected and PCR detectable and/or showing symptoms (which are typically around 3-5 days for COVID-19)³, and because of delays between illness onset and diagnosis. In Hong Kong, we estimated R_t with a 7-day lag by accounting for pre-symptomatic transmission and reporting delays^{4,5}.

An individual infected by SARS-CoV-2 will typically experience viral load peaking around illness onset and monotonically decreasing during the following two weeks⁶. While viral loads can vary across individuals, with some shedding more than others⁷, the mean distribution of viral loads from a group of patients measured around the time of illness onset will tend to have higher values than that of viral loads from a group of patients measured at a later time after infection⁸. Collectively, higher population-level viral loads would correlate with more infected persons being earlier in the course of infection and vice versa⁹. Viral loads can be proxied by cycle threshold (Ct) values in the real-time quantitative reverse-transcription polymerase chain reaction (RT-PCR) assay, with lower Ct values indicative of higher viral loads.

A recent study showed that the distribution of viral loads among confirmed cases can provide inferences on transmission dynamics within populations, where population-level Ct values skewing towards lower values indicate more individuals have been recently infected, corresponding to an increasing rate of epidemic growth in the community especially where single strain dominates⁹. The method was demonstrated in a modelling study using cross-sectional samples in a small-scale outbreak in Boston⁹ while the correlation was also observed over an epidemic wave elsewhere¹⁰. Here, we incorporated Ct values from COVID-19 cases in Hong Kong, a location with intense surveillance and case-finding efforts, to determine whether inclusion of data on Ct values could allow real-time estimation of transmissibility.

Results

In Hong Kong, COVID-19 cases are detected through clinical diagnosis for individuals with acute respiratory symptoms and public health surveillance for the community with a priority to people with pre-defined high exposure risks¹¹ (see Methods). Close contacts of confirmed cases are traced and placed into quarantine outside the home, and repeatedly tested. All laboratory-confirmed COVID-19 cases, including asymptomatic cases, are isolated within hospital and receive multiple RT-PCR tests during their stay. After excluding imported cases, we analyzed the first available record of Ct value for each confirmed case and characterized the daily distribution of Ct values (measured by mean and skewness) that were sorted by sampling days. We included two consecutive epidemics in July-August 2020 (i.e., the third wave) and November 2020 through March 2021 (i.e., the fourth wave), which were dominated by local transmissions instead of imported cases^{5,12}. Ct values were available for 96% (8268 out

of 8646) of local cases that were confirmed during the study period. The local COVID-19 vaccination program began in late February 2021 towards the end of the period studied. Variants of concerns (VoCs) were not reported among local cases during the study period, while two separate wild-type lineages dominated the two studied waves¹³.

We first examined the correlation between the distribution of daily Ct values and the local transmission dynamics⁵ (measured by the incidence-based R_t ; see Methods). The temporal Ct distribution tracked very closely the incidence-based R_t over the two epidemic waves (Fig. 1; Supplementary Fig. 1). Higher values of incidence-based R_t were found when the average Ct values decreased (Spearman's correlation coefficient, $\rho = -0.79$, $P < 0.001$ for the third wave and $\rho = -0.52$, $P < 0.001$ for the fourth wave) and when the Ct skewed towards lower values (i.e., greater values of skewness estimates; $\rho = 0.80$, $P < 0.001$ for the third wave and $\rho = 0.27$, $P < 0.001$ for the fourth wave) (Fig. 1; Supplementary Table 1).

To confirm that the changes in the observed daily Ct distribution were mostly driven by the epidemic dynamics despite individual variations in viral shedding, we extrapolated the Ct value back to illness onset for symptomatic cases by assuming that log-transformed Ct values increase after illness onset (see Methods). The skewness of the expected population-level Ct value distribution at illness onset (coefficient of variation: 0.37) was less variable than that of the observation at time of sampling (coefficient of variation: 0.80), suggesting a relatively stable peaking level of viral loads across individuals over the course of the epidemic (Supplementary Fig. 2).

To use Ct values for real-time assessing COVID-19 transmissibility in the community, we fitted a log-linear regression to daily incidence-based R_t on daily mean and skewness of Ct values at sampling during the third wave (i.e., training period; see Methods). We found that the distribution of Ct values explained 72% of the observed variations in incidence-based R_t during the training period (Supplementary Table 2). We then applied the trained model to the daily Ct distributions in the fourth wave (i.e., testing period) to estimate R_t in real time (i.e., Ct-based R_t). We found that the Ct-based method provided accurate real-time estimations of R_t during the 7-day lagged window suffered by the conventional incidence-based R_t estimation method (Fig. 2a). We found high correlations between Ct- and incidence-based R_t for both training (Spearman's correlation coefficient, $\rho = 0.81$, $P < 0.001$) and testing periods ($\rho = 0.48$, $P < 0.001$) (Fig. 2b-d). We conducted a sensitivity analysis to account for the potential impact of age on Ct distributions and therefore the Ct-based R_t , and found that the high correlation between Ct- and incidence-based R_t remained (Supplementary Fig. 3).

We performed a further validation of our results by training the model using data from November to December 2020 and predicting the third wave and found the high accuracy of predictions still held (Supplementary Fig. 4). We also performed a 10-fold cross-validation, in which we randomly assigned data between 6 July 2020 to 31 March 2021 into 10 validation sets. We found that on average 81% (ranging from 75% to 85%) of the Ct- and incidence-based R_t estimates were consistent (i.e., simultaneously below or above 1) across validation sets. These results suggested that relationships between Ct distributions and R_t estimates were not affected by temporal autocorrelation of incidence-based R_t . In addition, we found that our model predictions were not impacted by the length and timing of the training period as long as the training period covered

one complete epidemic wave (Supplementary Fig. 5). The key element of a suitable training period is to cover a period with sufficient samples to reflect epidemic changes, which usually starts from 2-3 weeks before the case peak till around 1-2 weeks after the peak (Supplementary Fig. 5). Longer training periods did not necessarily lead to better performance, possibly due to the variability in the longer tail with low numbers of samples (Supplementary Fig. 1 and 5).

Discussion

In this study, we applied a simplified Ct-based method to provide precise estimates of daily R_t and demonstrated that such a method could be used for real-time R_t estimation. Conventionally, the main challenge in estimating R_t in real-time was largely caused by the delays between an individual being infected and being PCR detectable or illness onset^{3,14}. Linking the incidence-based R_t and the population-level Ct distribution among samples collected on a given day was able to mitigate the right-censoring issue (i.e., missing cases that were infected but not-yet-observed due to the latent period) encountered by incidence-based methods^{1,3}. The few studies that have used population viral loads to infer COVID-19 epidemics only provided probability distributions of the estimated position of a community within an epidemic curve^{9,15}, while our method provides precise longitudinal R_t estimates, which further demonstrates the potential to improve real-time situational awareness using the Ct-based methods. In addition, we showed that the daily Ct distribution could be applied for tracking epidemics under a symptom and contact-tracing based setting, such as Hong Kong, providing empirical data to support the hypotheses generated from previous Ct-based studies⁹.

Our simplified Ct-based method also provides an approach for real-time estimation of R_t without requiring intensive surveillance of COVID-19 (i.e., accurate daily case counts by onset or diagnosis), which is of great significance especially for areas and time periods with limited surveillance capacity. Our results showed that population-level Ct distributions remained informative in tracking epidemic changes over time regardless of changes in surveillance in Hong Kong, especially by expanding the testing capacity during the fourth wave in our case¹¹. As the main prerequisite of the model was the distribution of Ct values among confirmed cases, our findings were less sensitive to changes in case reporting (e.g., due to definition changes and/or testing capacity constraints), which, by contrast, would greatly affect conventional incidence-based R_t estimation^{16,17}. For example, our Ct-based R_t indicated sustained community transmissions during the period between late October to early November 2020. This result is supported by R_t estimates generated from genomic data, which determined that SARS-CoV-2 introductions in September 2020, otherwise missed by incidence-based R_t methods, seeded undetected and sustained chains of transmission that eventuated in the fourth wave expansion detected in early November 2020¹³. Of note, R_t estimated with very few Ct samples (e.g., less than five) collected on a given day can lead to larger uncertainty, though we believe this may not be an issue for most areas with prevalent local COVID-19 transmissions, even if testing capacity is limited.

Our work is not the first attempt to improve real-time tracking COVID-19 transmissibility. Another study¹⁸ demonstrated that using sewage surveillance could shorten the prediction delays to two days ahead test positives, which, however, did not fully overcome the delays between being infected and being PCR detectable³. In addition, chances to locate sewage containing viral RNA could be low, especially when

transmission in the community was low. Under such situation, our method, which leverages existing information from confirmed cases, may be less resource-consuming and faster to implement, as long as the reporting delay could be shortened.

Future applications of our method may need adaptation to different populations, especially among those with which viral load trajectories differ. In particular, populations with higher SARS-CoV-2 vaccination rates may expect increased average Ct values when R_t is greater than 1, as lower viral loads were found in cases who had received COVID-19 vaccinations¹⁹. Similarly, increased Ct values when R_t is greater than 1 may also be found in populations younger than Hong Kong's population, due to the generally lower viral loads observed among young people⁶. As such, while we believe the intrinsic relationship between population viral loads and R_t estimates will remain valid (as long as the time relation between infection and viral shedding still holds), recalibrations of the model may be needed when applying our model to different populations.

By the time this study was performed, there were limited VoCs circulating in Hong Kong¹³, therefore we were not able to validate the generalizability of our model under outbreaks dominated by VoCs. A modeling study suggested that differences in population-level Ct values of samples from a symptom-based surveillance were more likely to reflect changes in viral load trajectories instead of differences in transmissibility across strains^{9,20}. As such, if increased viral loads (i.e., lower Ct values) occur with variant infections²¹, this may lead to decreased average Ct values when R_t is greater than 1. Leveraging information on factors affecting viral load levels (e.g.,

sequencing data) and regularly recalibrating the model will be necessary to update exact values of parameters for accurate nowcasting of R_t under more complex settings.

To summarize, in this analysis we applied a simplified method to incorporate the population-level viral loads into real-time estimation of transmissibility for COVID-19. We demonstrated that the Ct-based method could provide accurate nowcasting of R_t potentially allowing capacity-constrained regions to track local outbreaks quantitatively in a timely manner. Our method may need adaptations to different populations and the evolving strains, mainly to recalibrate the absolute extent to which the population viral loads correlate with COVID-19 transmissibility.

Methods

Study settings

Hong Kong was among the first places to identify COVID-19 cases globally, with its first COVID-19 case detected in late January 2020^{5,22}. Cases were classified as “imported cases”, “local cases epidemiologically linked with imported cases”, “unlinked local cases” and “local cases epidemiologically linked with local cases” according to their epidemiological characteristics and location of infection. All suspected COVID-19 cases were confirmed by RT-PCR in a local centralized public health laboratory. A laboratory-confirmed COVID-19 case was defined as a local case if the case did not visit places outside of Hong Kong in the 14 days before symptom onset (for symptomatic cases) or confirmation (for asymptomatic cases); otherwise defined as imported cases.

By the time of analysis, four different waves of transmissions have occurred in Hong Kong. In this study, we restricted our analyses to the third (July 2020 to August 2020)

and fourth (November 2020 to March 2021) waves which were dominated by local transmission, where 89% were local cases. Since we were interested in the local transmission of COVID-19, we only included local cases (unlinked local cases and local cases epidemiologically linked with local cases) in our analyses. Given the stringent border controls since July 2020²³ and the extreme small number of local cases linked with imported cases in Hong Kong (<0.1%), we assumed that all unlinked local cases were infected from other local cases. We did not include the first two waves (i.e., January to May 2020) as they were predominately imported cases and smaller clusters linked to those imported cases^{5,12}.

In Hong Kong, local COVID-19 cases were generally detected from clinical diagnosis that targeted people with acute respiratory symptoms and from public health surveillance that targeted population with predefined high risks of exposures (e.g., staff working at healthcare centers; residents living in neighborhood with any lab-confirmed cases) by health authorities¹¹. Upon case confirmation, contact tracing was carried out based on epidemiological information, with details described elsewhere²⁴. Among 8869 local COVID-19 cases confirmed between 1 July 2020 and 31 March 2021, 77% were detected symptomatic and 65% were found with epidemiological links with other known cases; across 23% of cases who were detected as asymptomatic, 81% of them were linked to other local cases. As such, the surveillance of COVID-19 in Hong Kong is largely symptom and contact-tracing based.

Data sources

Data on viral load of COVID-19 cases.

In Hong Kong, all confirmed COVID-19 cases (including asymptomatic cases) were admitted to hospitals for isolation and standardized management, with their hospitalization records stored in the data system managed by Hospital Authority (HA). Results for SARS-CoV-2 RT-PCR tests (LightMix® Modular SARS-CoV-2 (COVID-19) E-gene, TIB Molbiol/Roche, Berlin, Germany)²⁵ were recorded as Ct values in the system. The Ct value is the number of cycles needed to amplify the viral RNA in a specimen where the reported fluorescent signal reaches a pre-defined level in RT-PCR assays. Therefore, the Ct value is inversely associated with viral load, and could be used as a semi-quantitative measurement for viral load. In our main analysis, we used Ct values to measure viral load and analyzed the first recorded Ct value for each local case (which was usually sampled on or one day before admission) during the study period. Population viral load distributions were assessed by the date when samples were collected.

Demographic and epidemiological information of confirmed COVID-19 cases.

We obtained demographic and epidemiological information from the Department of Health of the Government of Hong Kong, including age, date of symptom onset and case classification (i.e., local, imported and contacts of local or imported cases).

Statistical methods

Estimating incidence-based R_t .

We estimated the incidence-based R_t for local cases using an extension of Cori et al^{4,26,27}. Briefly, local COVID-19 cases confirmed on each day t , $Q_1(t)$ was used for deconvolution to estimate the number of infections on each day t , $Y_1(t)$ ²⁸. We assumed an average 5.2 days (SD 3.9) for the incubation period¹⁴ and an average 4.7 days (SD 3.2

days, unpublished data) delay between illness onset to reporting empirically observed in Hong Kong, which were used for deconvolution. In this framework, the daily local R_t (i.e., the incidence-based R_t in our analysis) was the ratio between the number of new local cases at time t , $Y_1(t)$, and the total infectiousness of cases at time t , given by $\sum_{k=1}^{t-1} Y_1(k)w_L(t-k)$, where $w_L(t-k)$ denote the probability of being infectious $t-k$ days after infections. The transmission was modeled by a Poisson process, and therefore, we have

$$Y_1(t) \sim \text{Poisson}\{R_t \sum_{k=1}^{t-1} Y_1(k)w_L(t-k)\} \quad \text{Eq.1}$$

$w_L(t-k)$ was estimated using the convolution of the incubation period (mean 5.2 days, SD 3.9)¹⁴ and the infectiousness relative to onset² (details described elsewhere⁴). To fully utilize available case count information and to provide more timely R_t estimates under the incidence-based method, we used the smoothing method described in Cori et al²⁷ and calculated R_t estimates over a time window of size $\tau = 14$ ending at time t , assuming that the transmissibility was constant over the time period $[t-\tau+1, t]$. We used a data-augmented Markov chain Monte Carlo algorithm to estimate the incidence-based R_t . To account for the uncertainty of other parameter such as the incubation period, we used an bootstrap approach in Salje et al²⁹ to reconstruct 200 epidemic curves and perform estimation. After that we presented the mean, 2.5% and 97.5% quantiles for those 200 R_t estimates for each day t . More details about incidence-based R_t estimation was described elsewhere⁴.

Temporal distribution of population-level Ct values.

We analyzed the first available Ct value record for each local COVID-19 case (i.e., $y_{j,t}$, t is the calendar date when the first sample was collected for individual j). To characterize

the temporal distribution of population-level Ct values over the study period, we fitted a generalized additive model (GAM) to the above-mentioned data over calendar time:

$$y_{j,t} = \alpha_0 + s(t) \quad \text{Eq.2}$$

where $s(t)$ was the smooth function for date t over study period. 95% confidence intervals (CIs) of the smoothed average daily Ct were derived from 500 bootstraps (Fig. 1b). In each bootstrap, we resampled from the data on cases' first available Ct values and refitted the GAM. We did not include samples collected between 1 September 2020 to 31 October 2020 due to the small number of samples that were collected on each day.

To validate that the observed temporal variations in population-level Ct distribution was not driven by variations in individual viral load trajectories, we estimated the Ct value on the date of illness onset (Supplementary Fig. 2). We fitted a log-linear regression of the first available Ct value for individual j (y_j) on the time interval between the individual's illness onset and first sample collection (δ_j) and age group (a_j , modelled as categorical, i.e., 0-18, 19-64 and ≥ 65 years old):

$$\ln(y_j) = \beta_j + \beta_1 \delta_j + \beta_2 a_j + \beta_3 \delta_j a_j \quad \text{Eq.3}$$

where β_1 , β_2 and β_3 are the estimated coefficients for the time interval between the illness onset and first sample collection, age group and their interaction, respectively. We then calculated the back-projected Ct value at illness onset by setting $\delta_j = 0$. We chose the log-linear model as the Akaike information criterion (AIC) indicated it outperformed the linear model in terms of model fit (-9177 and 38279 for the log-linear and linear models, respectively).

We found that the skewness of Ct values at sampling was more variable (coefficient of variation: 0.80) than the skewness of Ct values extrapolated at onset (coefficient of

variation: 0.37) (Supplementary Fig. 2), suggesting that variations in individual viral load trajectories may not be the major driver of the observed temporal variation in population-level Ct distribution over our study period during which only the wild-type SARS-CoV-2 strains have been circulating locally.

Incorporating Ct distributions into R_t estimation (Ct-based R_t).

We used the mean ($\bar{x}_t$) and skewness (b_t)³⁰ to measure the distribution of Ct values that were sampled on date t :

$$\bar{x}_t = \frac{1}{n_t} \sum_{i=1}^{n_t} y_{t,i} \quad \text{Eq.4}$$

$$b_t = \frac{\frac{1}{n_t} \sum_{i=1}^{n_t} (y_{t,i} - \bar{x}_t)^3}{\left[\frac{1}{n_t - 1} \sum_{i=1}^{n_t} (y_{t,i} - \bar{x}_t)^2 \right]^{\frac{3}{2}}} \quad \text{Eq.5}$$

where $y_{t,i}$ represented the i th ($i = 1, 2, \dots, n_t$) of the total n_t Ct values that were sampled on day t . 95% CIs of the daily skewness b_t were calculated from 500 bootstraps (Fig. 1c), with data on cases' first available Ct values resampled in each bootstrap to recalculate the daily skewness.

We first calculated the Spearman's rank correlation coefficient (ρ) between daily Ct distribution (i.e., daily mean and skewness) and the natural log-transformed incidence-based R_t (Supplementary Table 1).

To determine the best fit model that characterized the association between daily Ct distribution and the incidence-based R_t , we compared AIC of a series of regression models over the training period (i.e., 6 July 2020 to 31 August 2020), which used different formats of dependent variable and measurements for predictive variables

(Supplementary Table 3). We compared models that were fitted to linear scale and natural log-transformed incidence-based R_t . We also assessed models that included different combinations of measurements for daily Ct distributions, including mean, median and skewness. We imputed the daily Ct distributions using the average of that within the preceding 7 days when no samples were collected on that day. The model fitted to natural log-transformed incidence-based R_t ($\ln(R_t)$) on daily mean ($\bar{x}_t$) and skewness (b_t) of Ct values was found with the lowest AIC and was used in our main analyses (Supplementary Table 3):

$$\ln(R_t) = \gamma_0 + \gamma_{\bar{x}}\bar{x}_t + \gamma_b b_t \quad \text{Eq.6}$$

where $\gamma_{\bar{x}}$ and γ_b were coefficients for daily mean and skewness of Ct values from the regression model and were reported in Supplementary Table 2 after exponential transformation.

To assess whether our results would be affected by the age distribution of cases who were sampled on each day, we performed a sensitivity analysis by including the mean age ($\bar{a}_t$) of cases whose first sample were collected on day t into the above-mentioned main model (Eq. 6):

$$\ln(R_t) = \gamma_0 + \gamma_{\bar{x}}\bar{x}_t + \gamma_b b_t + \gamma_{\bar{a}}\bar{a}_t \quad \text{Eq.7}$$

Results suggested similar predictions from models with and without considering cases' age distribution (Supplementary Fig. 3).

Cross-validations of the model.

To validate the generalizability of this Ct-based method, we fitted the main model (Eq. 6) using data from an alternative training period, i.e., from 20 November 2020 to 31

December 2020 (the initial stage of the fourth wave) (Supplementary Fig. 4, Supplementary Table 2).

We further performed a 10-fold cross-validation by randomly splitting the data between 6 July 2020 and 31 March 2021 into ten validation sets, after excluding days when less than five available Ct samples were collected. For each validation, we held one set as testing set, and trained the rest nine sets using the main model (Eq. 6). We compared the consistency between the Ct- and incidence-based R_t for the testing set by calculating the proportion of days when the two estimates were simultaneously below or above 1 (i.e., in the same direction) over total duration of each validation set. We also assessed the prediction performance using the mean absolute error (MAE) for the Ct- ($E(R_t)$) and incidence-based R_t for each validation set:

$$MAE = \frac{\sum_{d \in D} |\ln(E(R_d)) - \ln(R_d)|}{N_D} \quad \text{Eq.8}$$

where d is a given date in the validation set D and N_D is the number of days included in each validation set. We found an average of 0.28 (ranging from 0.25 to 0.34) of the MAE across ten validation sets, suggesting a good performance of our model predictions.

Data availability

All the data supporting the findings of this study are available and included in this article and its supplementary information files.

Code availability

The source code will be available after publication in the GitHub repository (https://github.com/vanialin/ct_rt_hk).

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Author contributions

All authors meet the ICMJE criteria for authorship. The study was conceived by BJC and BY. YL, EHYL, JYTW, HSB, JKC, FH, HG and TKT prepared the data. BY and YL developed the model. YL and BY conducted the data analyses. SC, DCA, STA, NHLH, TKT, PW, GML and BJC interpreted the results. YL and BY wrote the first draft of the manuscript. All authors provided critical review and revision of the text and approved the final version.

Competing Interests Statement

BJC consults for AstraZeneca, GSK, Moderna, Roche and Sanofi Pasteur. The authors report no other potential conflicts of interest.

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Figure Legends

Fig. 1 Correlations between temporal distribution of Ct values and transmission dynamics of COVID-19 in Hong Kong. **a**, Local COVID-19 cases and the estimated incidence-based R_t . Grey bars indicate the number of laboratory-confirmed local cases by date of reporting. Black lines and shaded areas indicate the mean and 95% credible intervals (CrIs) for incidence-based R_t . **b**, Ct distributions smoothed from a generalized additive model (GAM). Dark grey bars indicate the number of sample collections. Orange lines and shaded areas indicate the daily average and 95% confidence intervals (CIs) of Ct values that were estimated from a GAM (Eq. 2) over the study period in Hong Kong. **c**, Daily skewness of Ct values over the study period. Blue dots represent the observed daily Ct skewness and vertical lines represent 95% CIs of daily Ct skewness that were calculated from 500 bootstraps. **d-e**, Correlations between the daily incidence-based R_t and the daily mean Ct (**d**) or skewness (**e**). Boxes represent the interquartile and median of the incidence-based R_t .

Fig. 2 Nowcast of the transmission dynamics of COVID-19 using Ct distribution. **a**, Nowcasting R_t using the Ct-based method over four representative weeks. Grey bars represent the number of laboratory-confirmed local cases by date of reporting. Black lines and shaded areas indicate the mean and 95% CrIs for incidence-based R_t (same for **b-c**). Pink dots and vertical lines represent point estimates and 95% prediction intervals for Ct-based R_t estimated from Eq. 6 (same for **b-c**). **b-c**, Comparison of incidence-based R_t and Ct-based R_t over the training period (July 2020-August 2020, **b**) and the testing period (November 2020-March 2021, **c**). **d**, Distributions of incidence-based R_t under various intervals of Ct-based R_t . Boxes represent the interquartile and median of incidence-based R_t under the corresponding interval of Ct-based R_t over training (purple) and testing (pink) periods.

Supplementary Figure 1. Daily distribution of Ct values by sampling date over the study period. Dark grey vertical lines and red dots showed the interquartile and median of Ct values sampled per day, while light grey vertical lines represented either the minimum and maximum of all Ct values or the smallest and largest observed Ct values that are within the distance of 1.5 times the interquartile range of all Ct values on that day (i.e., whiskers). Orange lines and shaded areas represent the daily average and the 95% CI of Ct values estimated from a GAM as in Eq. 2 (same as Fig. 1).

Supplementary Figure 2. Bi-weekly distribution of Ct values by date of sampling and of onset during the third (a) and fourth (b-c) wave of COVID-19 in Hong Kong. The Ct value shown by date of sampling was the actual value as sampled, whereas the Ct value shown by date of onset was the extrapolated value estimated from Eq. 3. The distribution of Ct values at sampling and at onset was illustrated in green and orange if during periods with increasing epidemic and in cyan and pink if during periods with decreasing epidemic. The skewness of Ct distribution at sampling (b_s) and at onset (b_o) over the biweekly period was shown at top-right of each panel with corresponding colors, while the exact time period was specified above each panel.

Supplementary Figure 3. Consistent Ct-based R_t estimates before and after adjusting for the age distribution of sampled cases. a-b, Comparison of daily mean Ct (a) and Ct skewness (b) under various incidence-based R_t intervals during July 2020 - August 2020 (wave 3) and November 2020 - March 2021 (wave 4). Boxes show the interquartile and median of the corresponding Ct distribution derived from all samples (colored) and from samples of adult cases only (white) respectively. c) Comparison of Ct-based R_t estimates before and after adjustment of age. Pink lines and shaded areas show the point estimates and 95% prediction intervals of Ct-based R_t estimated from the main model (Eq. 6), while blue dots and vertical lines show the point estimates and 95% prediction intervals of Ct-based R_t estimated after adjusting for the mean age of daily sampled cases (as in Eq. 7).

Supplementary Figure 4. Validation of the Ct-based model using an alternative training period. a-b, Comparison of incidence-based and Ct-based R_t over the alternative training (November-December 2020, a) and testing (July-August 2020, b)

periods. Black lines and shaded areas indicate the mean and 95% CrIs for incidence-based R_t , while colored dots and vertical lines represent the point estimates and 95% prediction intervals of Ct-based R_t . **c)** Consistency between the incidence-based and Ct-based R_t over the alternative training (purple) and testing (pink) periods.

Supplementary Figure 5. Performance of models fitted over various length and timing of training periods. a-b, Performance of models fitted over different training periods with various durations and starting dates, over **a)** July-September 2020 and **b)** November 2020-January 2021. Color lines show the performance of models (measured by adjusted R square) fitted over training periods with a duration of 30-60 days respectively, with the starting date of the training period specified in the x-axis. The grey dashed line in the background referred to adjusted R square being 0.7, as a reference for comparison. **c-d,** Time period covered by the model with the largest adjusted R square over **c)** July-September 2020 and **d)** November 2020-January 2021. Grey and orange bars represent the number of laboratory-confirmed cases (by date of reporting) and of sampled collections (by date of sampling) respectively. Time periods used for fitting the model with the largest adjusted R square among all models fitted over that certain length of training period was indicated by corresponding colored backgrounds (same color legend as in panel **a**).

Figures

Figure 1

Correlations between temporal distribution of Ct values and transmission dynamics of COVID-19 in Hong Kong. a, Local COVID-19 cases and the estimated incidence-based R_t . Grey bars indicate the number of laboratory-confirmed local cases by date of reporting. Black lines and shaded areas indicate the mean and 95% credible intervals (Cris) for incidence-based R_t . b, Ct distributions smoothed from a generalized additive model (GAM). Dark grey bars indicate the number of sample collections. Orange lines and shaded areas indicate the daily average and 95% confidence intervals (CIs) of Ct values that were estimated from a GAM (Eq. 2) over the study period in Hong Kong. c, Daily skewness of Ct values over the study period. Blue dots represent the observed daily Ct skewness and vertical lines represent 95% CIs of daily Ct skewness that were calculated from 500 bootstraps. d-e, Correlations between the daily incidence-based R_t and the daily mean Ct (d) or skewness (e). Boxes represent the interquartile and median of the incidence-based R_t .

Figure 2

Nowcast of the transmission dynamics of COVID-19 using Ct distribution. a, Nowcasting R_t using the Ct-based method over four representative weeks. Grey bars represent the number of laboratory-confirmed local cases by date of reporting. Black lines and shaded areas indicate the mean and 95% CIs for incidence-based R_t (same for b-c). Pink dots and vertical lines represent point estimates and 95% prediction intervals for Ct-based R_t estimated from Eq. 6 (same for b-c). b-c, Comparison of incidence-based R_t and Ct-based R_t over the training period (July 2020-August 2020, b) and the testing period (November 2020-March 2021, c). d, Distributions of incidence-based R_t under various intervals of Ct-based R_t . Boxes represent the interquartile and median of incidence-based R_t under the corresponding interval of Ct-based R_t over training (purple) and testing (pink) periods.

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