**Title**: High transmission rates of early omicron sub-variant BA.2 in Bangkok, Thailand

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**Abstract [158 words]**:

Omicron emerged as the fifth variant of concern of SARS-CoV-2 coronavirus pandemic in late 2021 and rapidly overtook the previously predominant Delta variant~~s~~ with a significantly faster transmission rate and unique mutations on the spike gene. Hence, the ability to identify viral variants rapidly and affordably in large number of patients, which facilitates the monitoring of the transmission and clinical impact of new variants, is needed to obtain information for updating the public health policy. In this study, we evaluated the capability of two RT-PCR and mass spectrometry-based SARS-CoV-2 variant classification platforms to distinguish Delta, Omicron BA.1, and Omicron BA.2 variants in 618 COVID-19-positive samples from patients in Bangkok collected during November 2011-March 2022. Analysis of the time-evolution pattern of SARS-CoV-2 variant profiles indicated that the BA.1 and BA.2 possess up to 2-3 times higher transmission rates than the Delta variant. Our study showcases a cost-effective virus surveillance that enables a quantitative estimation of variant-specific public health impact.

**Keywords:**

SARS-CoV-2, transmission rate, variant classification

**Introduction**

Omicron emerged as the fifth variant of concern (VOC) of coronavirus disease (COVID-19) in November 2021, replacing the predominant Delta variants. Omicron was first identified on November 11, 2021, in Botswana and on November 14, 2021, in South Africa. 1 Omicron contains more than 30 mutations on its spike protein, including 15 mutations in the receptor-binding domain (RBD) that might underlie its increased transmissibility and reduced vaccine efficacy. 2 In April 2022, the World Health Organization (WHO) announced the BA.1, BA.2, BA.3, BA.4, and BA.5 Omicron sub-variants for surveillance. 3 Hence, the ability to detect these new variants is required to monitor their spread, evaluate their clinical impact, and update public health policy.

The most common lineages of Omicron in early 2022 were BA.1 (B.1.1.529.1), BA.2 (B.1.1.529.2), and BA.3 (B.1.1.529.3). These variants share 12 mutations in the RBD which binds to human angiotensin-converting enzyme 2 (ACE2) proteins and is responsible for viral entry into the host cell. 4 Additionally, these variants also share 21 common mutations in other regions of the spike protein, such as the N501Y and Q498R mutations that are expected to enhance the binding to ACE2 receptors and the H655Y, N679K, and P681H mutations that are believed to increase spike cleavage and facilitate virus transmission. 5 BA.2 is of particular interest because it was reportedly 1.5-fold more infectious than BA.1 and 4.2-time more than Delta. BA.2 has a 30% higher potential than BA.1 to escape existing vaccines and is 17-fold more capable than Delta 6 in this regard. BA.2 is 35-fold more resistant to sotrovimab, a monoclonal antibody, compared to the ancestral D614G-bearing B.1.1 virus. Moreover, BA.2 is 6.4-fold more resistance than BA.1 in neutralization assay using murine sera. 7 BA.2 contains S371F, T376A, D405N, and R408S substitutions in the RBD, which might increase its rate of spread, 8 along with unique mutations, T19I, L24S, P25del, P26del, A27S, V213G, T376A, and R408S. 4

In Thailand, Omicron is the fifth wave of COVID-19 pandemic that started around January 2022 and spread much faster than the earlier Delta variants. 9 This situation prompted our team, the Thai Red Cross Emerging Infectious Diseases Clinical Center (TRC-EIDCC), to develop a cost-effective and rapid workflow for classifying SARS-CoV-2 variants in patients who visited the King Chulalongkorn Memorial Hospital (KCMH). In this study, we compared whole-genome sequencing, which is the gold standard method for SARS-CoV-2 variant classification, to more affordable array-based (Novaplex™ SARS-CoV-2 Variants VII) and mass spectrometry-based methods (MassARRAY®). The collected data let us derive an estimate for the increased transmission rate of the Omicron variants compared to the Delta variant that is consistent with estimates obtained from GISAID data. 10 Hence, the ability to detect viral variants using affordable technology can enable a sentinel surveillance site to quantitatively monitor and evaluate the impact of an outbreak.

**Methods**

**Swab sampling and viral RNA extraction**

Oropharyngeal swabs of suspected COVID-19 patients were collected between 5 November 2021 and 31 March 2022 as a part of routine SARS-CoV-2 surveillance at the TRC-EIDCC from KCMH (IRB=No. 361/59, 400/63), Suvarnabhumi Airport (Division of International Communicable Disease Control Port and Quarantine) and other organizations in Bangkok. Viral RNA was extracted from the samples using a MagPurix® 12 EVO automated Nucleic Acid purification system (Zinext Life Science Corp) and confirmed for SARS-CoV-2 by reverse transcription PCR (RT-qPCR) test.

**SARS-CoV-2 variant classification**

As mentioned, three methods were used to classify SARS-CoV-2 variants in positive samples, namely Novaplex™ SARS-CoV-2 Variants VII Assay (Novaplex) (Seegene Technologies), MassARRAY® System (Agena Bioscience), and whole genome sequencing (WGS) using Next-Generation Sequencing (NGS)(Illumina).

For Novaplex, the detection of E484A and HV69/70 deletion in spike gene, N501Y in RdRP gene, and endogenous internal control were performed according to the manufacturer’s instructions on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The test results were analyzed with Seegene software using a positive cut-off of Ct<42. The list of targeted mutations is provided in Table 1.

For MassARRAY® System, a multiplex PCR MassARRAY assay (PMA) were conducted using specific point mutation panels. Four different point mutation panels of PMA were designed based on the circulating variants and used as the assay throughout the period, namely, ABDO V1, Omicron V1, Omicron V2 and Omicron V3 (Table 1). Samples with Ct<30 were analyzed with RT-PCR using iPLEX pro chemistry reagent for target regions amplification and MALDI-TOF mass spectrometer (MassARRAY Analyzer) 11 to detect nucleotide at target mutations of each panel

For WGS, viral RNA was amplified by ARTIC V3 and V4 protocols. DNA library was prepared using an Illumina® DNA Prep kit with Respiratory Virus Oligos Panel v2 (Illumina) enrichment. Sequencing was performed on a MiSeq platform using a 2 x 250 nucleotides reagent kit v2 and assembled by mapping with the reference genome Wuhan-Hu-1 (NC\_045512.2) as previously described in 12. Variant of the genomes were classified using Pangolin 13 and Nextclade 14.

**Estimation of the transmission rates for each variant**

The number of new cases at time *t* + 1, *Nt*+1, were modeled using three factors, the current number of cases, *Nt*, the current fractional abundance of each variant*,* {*ft*Delta, *ft*BA.1, *ft*BA.2} and the transmission rate of each variant {*r*Delta, *r*BA.1, *r*BA.2}, which represents the number of new cases that could arise from an infected person over a period of time and is assumed to be time-independent:

Here, a unit of time was set at 5 days. A first-order competition model was used to estimate the dynamics of the fractional abundance of viral variants:

The search for the best-fitted transmission rate of each variant {*r*Delta, *r*BA.1, *r*BA.2} was performed using SciPy’s minimize function with weighted mean squared error (weighted by the number of tested samples at each time point) as the objective. To estimate the variability of the fitted transmission rates, the parameter fitting process was repeated on 100 random initial guesses for the transmission rates, each drawn uniformly from [0, 1], and 100 bootstrap sampling of the time-series daily case data, each drawn from two-third of the number of time points without replacement. The numbers of new cases in Bangkok during the time period were collected from Thailand Ministry of Public Health record. The fractional abundances of the Delta, BA.1, and BA.2 variants in Bangkok during the time period were estimated based on either only our local samples or submitted entries on GISAID.

**Results**

The TRC-EIDCC identified the first Omicron case (BA.1) from a sample from Suvarnabhumi airport on 8 December 2021, when the number of daily new case in Thailand was around 3,000-4,000 cases. Then, the first Omicron BA.2 case was detected on 8 January 2022, when the number of daily new cases has reached 10,000. As shown in Figure 1, the new Omicron variants quickly replaced the prevalent Delta variant in early-January, although some Delta cases can still be found up until early March. The BA.2 lineage then replaced BA.1 as the most dominant lineage in early-March. Similar relative abundances of the three variants of interest, Delta, Omicron BA.1, and Omicron BA.2 were obtained with either GISAID data (n = 4,295 for Bangkok and n = 11,422 for Thailand) or our cohorts (n = 612).­­­­

From 5 Nov 2021 to 31 March 2022 (21 weeks), a total of 618 samples tested positive for SARS-CoV-2 were analyzed at our center using three assays, namely Novaplex, PMA, and WGS, to identify SARS-CoV-2 variants. Out of 618 samples, 261 were subjected to multiple assays and only nine were discordant (Table 2). All discordant results were due to Novaplex’s limited ability to detect mutations.

To estimate the transmission rates, i.e., the number of new infections that could arise on average from an infected individual, a linear model linking the relative abundance and the transmission rate of each variant to the number of daily cases was built (see Methods). The estimation process was repeated 100 times with different random initial guesses to determine the uncertainty. As shown in Figure 2a, despite small sample counts, data from our local cohorts (n = 612) yielded a similar estimate as Bangkok data from GISAID (n = 4,295). The transmission rate for Omicron BA.1 was estimated to be 2.23 (SD = 0.22) and 2.09 (SD = 0.14) times that of the Delta variant, while the transmission rate for Omicron BA.2 was estimated to be 3.38 (SD = 0.43) and 3.29 (SD = 0.24) times that of the Delta variant. Interestingly, using Thailand data from GISAID yielded significantly lower estimates of 1.78 (SD = 0.18) for BA.1 relative to Delta and 2.67 (SD = 0.38) for BA.2 relative to Delta, respectively (Mann-Whitney U test p-values < 3e-24). The baseline transmission rate for the Delta variant was estimated to be 0.58 (SD = 0.06), 0.59 (SD = 0.04), and 0.66 (SD = 0.06) using local data, GISAID data for Bangkok, and GISAID data for Thailand, respectively. In all cases, these estimates fit well to the observed abundances and case counts (Figure 2b).

**Discussion**

The Omicron BA.1 variant (B.1.1.529) rapidly replaced the predominant Delta strain within 4 weeks, leading to the fifth wave of COVID-19 in Thailand (Figure 1). The rapid spread of Omicron was similar across countries, however, the immunity from infection and vaccination differed, such as the cases in Denmark, 8 South Africa, 15 and EU. 16 Differences in mutations on the spike protein of Omicron BA.1 and BA.2 may explain their high transmissibility. BA.2 has deletions at amino acid positions 24-26 and A27S substitution, whereas BA.1 has deletions at amino acid positions 69-70 and 142-144. These positions are located near the N-terminal domain (NTD) antigenic site and are associated with resistance to neutralizing monoclonal antibodies. 17 The deletion at amino acid position 69-70 in spike protein affects the antigenicity leading to resistance against neutralizing antibodies and defines the sub-lineages BA.1 and BA.2. 18

The Novaplex™ assay is easy-to-use, fast, cost-effective, and able to handle low-concentration samples (Ct<42). However, this assay can detect only three point mutations, E484A, HV69/70 deletion, and N501Y, which are insufficient for distinguishing existing variants, such as between Delta and Delta plus. On the other hand, the PMA platform can accommodate up to 40 point mutations, producing more information for classifying sub-variants. Furthermore, PMA utilizes PCR and mass spectrometry which are not as expensive as WGS and is applicable to samples with lower viral loads (Ct<35 compared to Ct<26 for WGS). 11 Although WGS is still a gold standard method for variant classification and novel variant identification, PMA and Novaplex™ can be beneficial for screening variants in the high transmission areas and for pre-selecting samples for WGS. In particular, the choices of 40 point-mutations in PMA can be continually updated to encompass new variants, as done in this study (Table 1). These assays are also highly concordant (96.5%, 252 out of 261 cases).

Even with limited number of samples (n = 612), the variant abundances observed in our local cohorts still yield consistent estimates of the transmission rates compared to using the much larger datasets from GISAID (Figure 2a). This allowed us to robustly estimate the trend of variant abundances as well as case counts (Figure 2b). The lower transmission rates estimated using data from all over Thailand compared to Bangkok data fit the expectation that higher transmissibility would be observed in densely populated areas, like Bangkok, compared to more rural areas. However, it should be noted that external factors, such as the saturation of PCR testing capacity, under-reporting of new cases, and changes in public health policy can confound the observations.

**Conclusion**

The use of the affordable mass spectrometry-based MassARRAY® System for detecting SARS-CoV-2 variants in clinical samples enabled sentinel surveillance at a primary health care institution. This method is also flexible, allowing primer customization to target new emerging mutations, and has a rapid turnaround time. The ability to monitor and predict the current magnitude of infection and change in transmission rate using our strategy facilitates prompt allocation of vaccines and treatment resources that prevents overburden of hospital admission.

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**Data availability**

SARS-CoV-2 whole genome sequences generated in this study are deposited into the GISAID repository (<https://www.gisaid.org>). GISAID ID and the SARS-CoV-2 variant classification results of 618 positive SARS-CoV-2 samples analyzed by three methods are provided in supplementary Table S1. New daily SARS-CoV-2 cases in Bangkok and Thailand were retrieved from the Thailand Department of Disease Control COVID-19 API (<https://ddc.moph.go.th/covid19-daily-dashboard/>), the data is also provided in supplementary Table S2.

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**Table**

**Table 1**: Mutations targeted by Novaplex™ SARS-CoV-2 Variants VII Assay and three versions of PMA MassARRAY panels.

|  |  |  |
| --- | --- | --- |
| Method | Mutation | Control |
| Novaplex™ SARS-CoV-2 Variants VII Assay | **S gene**: HV69del, E484A, N501Y | RdRp gene |
| MassARRAY® ABDO V1a | **S gene**: S13I, T20N, A67V, HV69del, D80A, Y144del, W152C, F157L, R190S, LLA241del, D253G, V367F, K417N, K417T, L452R, E484Q, E484K, N501Y, A570D, Q613H, D614G, H655Y, Q677H, P681R, P681H, A701V, T716I, F888L, S982A, T1027I, E1092K, H1101Y, V1176F, D1118H | **N gene**: C29208del |
| MassARRAY® Omicron V1a | **S gene**: T19R, T19I, A67V, HV69del, T95I, GVY142del, N211del, G339D, S373P, S375F, K417N, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, T574K, D614G, H655Y, P681R, P681H, D796Y, N856K, Q954H, N969K, L981F, D1118H |
| MassARRAY® Omicron V2a | **S gene**: T19R, T19I, A67V, HV69del, T95I, GVY142del, N211del, G339D, R346K, S373P, S375F, K417N, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, T547K, D614G, H655Y, P681R, P681H, D796Y, N856K, Q954H, N969K, L981F, D1118H, I1221T |
| MassARRAY® Omicron V3a | **S gene**: V3G, T19I, T19R, A67V, HV69del, T95I, G142D, K147E, G339H, G339D, R346K, K417N, L452R, N460K, S477N, T478K, E484A, F486V, Q493R, Q498R, N501Y, Y505H, T547K, D614G, H655Y, P681H, P681R, D796Y, N856K, Q954H, L981F, I1221T,  **N gene**: P151S, ORF7b: L11F |

a Different mutation panels were used at different time periods as the assay was continually improved. ABDO V1 was used until 16 December 2021. Omicron V1 was used from 18 December 2021 until 3 January 2022. Omicron V2 was used from 4 January 2022 until 28 March 2022. Omicron V3 was used from 27 March 2022 onwards.

**Table 2**: Number of positive samples detected by each assay combination.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Novaplex | PMA | WGS | Samples | Mismatchesb |
| x |  |  | 68 |  |
|  | x |  | 279 |  |
|  |  | x | 4 |  |
| x | x |  | 137 | 8 |
| x |  | x | 48 | 1 |
|  | x | x | 35 | - |
| x | x | x | 41 | - |
|  |  |  | 6a |  |
| Total | | | 618 | 9 |

a Samples that failed variant classification step, two samples from each method.  
b Mismatch results, different variants detected by multiple methods, were cleaned before data analysis by basing the result on the more reliable method, ranging from the gold standard WGS, PMA and Novaplex.

**Figure and captions**



**Figure 1**: **The trends of daily new cases and relative abundances of the Delta and Omicron variants from Nov 2021 to Mar 2022 in Thailand, Bangkok, and our hospital**. Variant abundances data for Thailand and Bangkok were retrieved from GISAID. Numbers of daily new cases were retrieved from the report released by Thailand Ministry of Public Health. Data were smoothed with a 5-day average sliding window.



**Figure 2**: **Estimated transmission rate ratios for Omicron BA.1 and BA.2 variants relative to the Delta variant (a) and goodness of fit between estimated transmission rates and observations (b)**. a) Variant abundance data from local cohorts, GISAID entries for Bangkok, and GISAID entries for Thailand were used. The distributions of the rate ratios were estimated from 100 optimization repeats with different randomly initialized values (see Methods). Mean values were denoted by orange bars. Boxes indicate the interquartile ranges. Whiskers indicate the 1.5x ranges below the first and above the third quartiles. Circles denote outliers. b) Scatter plots show the agreement between observed variant frequencies and case counts versus the predictions based on estimated transmission rates. Each data point corresponds to a 5-day period during Nov 2021 to Mar 2022. Results for the estimated transmission rates with the lowest mean square error on data from our hospital are shown.