

# High concordance in SARS-CoV-2 detection between automated (*Abbott m2000*) and manual (*DaAn gene*) RT-PCR systems: The EDCTP PERFECT-Study in Cameroon

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## Abstract

Molecular diagnosis of COVID-19 is critical to the control of the pandemic, which is a major threat to global health. Several molecular tests have been validated by WHO, but would require operational evaluation in the field to ensure their interoperability in diagnosis. In order to ensure

field interoperability in molecular assays for detection of SARS-CoV-2 RNA, we evaluated the diagnostic concordance of SARS-CoV-2 between an automated (*Abbott*) and a manual (*DaAn gene*) real-time PCR (rRT-PCR), two commonly used assays in Africa. A comparative study was conducted on 287 nasopharyngeal specimens at the Chantal BIYA International Reference Centre (CIRCB) in Yaounde-Cameroon. Samples were tested in parallel with *Abbott* and *DaAn gene* rRT-PCR, and performance characteristics were evaluated by Cohen's coefficient and Spearman's correlation. A total of 273 participants [median age (IQR) 36 (26-46) years] and 14 EQA specimens were included in the study. Positivity was on 30.0% (86/287) *Abbott* and 37.6% (108/287) *DaAn gene*. Overall agreement was 82.6% (237/287), with  $k=0.82$  (95%CI: 0.777-0.863), indicating an excellent diagnostic agreement. The positive and negative agreement was 66.67% (72/108) and 92.18 % (165/179) respectively. Regarding Viral Load (VL), positive agreement was 100% for samples with high VLs (CT<20). Among positive SARS-CoV-2 cases, the mean difference in Cycle Threshold (CT) for the manual and Cycle Number (CN) for the automated was  $6.75\pm 0.3$ . The excellent agreement (>80%) between the *Abbott* and *DaAn gene* rRT-PCR platforms supports interoperability between the two assays. Discordance occurs at low-VL, thus underscoring these tools as efficient weapons in limiting SARS-CoV-2 community transmission.

## Introduction

Coronaviruses are a large family of viruses, which may cause disease in animals or humans. Seven coronaviruses can lead to infection, but four of them are most common in human, namely 229E, NL63, OC43, and HKU1.<sup>1</sup> They usually cause a wide range of respiratory infections varying from the common cold to more severe diseases such as the Middle East Respiratory Syndrome (MERS) and the Severe Acute Respiratory Syndrome (SARS) of which the most recently discovered is the SARS-CoV-2, responsible for the coronavirus disease in 2019 (COVID-19).<sup>1,2</sup> Time from exposure to SARS-CoV-2 and symptom onset is generally between 2-14 days, with an average of 5 days. Common symptoms include fever, cough, sneezing and shortness of breath. Complications may include pneumonia, throat pain, acute respiratory distress syndrome, cytokine storm, blood clotting, and even death.<sup>2,3</sup>

As of February 23<sup>th</sup> 2022, there have

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Key words: Molecular diagnosis; SARS-CoV-2; rRT-PCR; Concordance; Cameroon.

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Informed consent: Informed informed consent was obtained.

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been 426,624,859 confirmed cases of COVID-19 worldwide, including 5,899,578 deaths with a total of 10,407,359,583 vaccine doses administered,<sup>4</sup> with the USA reporting the highest number of cases all around the world.<sup>4</sup> In Africa, 53 countries were declared affected with 11,129,305 cases and 247,310 deaths. Of all African countries, South Africa still records the highest number of cases.<sup>5</sup> In Cameroon, we recorded 119,107 confirmed cases on February 16<sup>th</sup>, 2022 including 1,920 deaths and 1,066 951 received at least one dose of vaccine (representing 7.7% of the target population). With a case fatality rate of 1.6%, the country remains among the African countries with the highest number of infections.<sup>6</sup>

To ensure an effective control and surveillance system of COVID-19, the World Health Organization (WHO) provides Emergency Use Authorization (EUA) to molecular and non-molecular techniques for the diagnosis of SARS-CoV-2 infection. Non-molecular techniques such as rapid diagnostic tests, involving antigens and antibodies have been authorized for the large scale detection of SARS-CoV-2 and sero-surveillance within the context of massive spread of the pandemic.<sup>7-9</sup> Although non-molecular techniques are very effective (high specificity) in slowing the spread, they are also less sensitive in identifying cases with low-level viremia as compared to molecular diagnostics.<sup>10</sup> Interestingly, molecular assays involving real-time amplification (rRT-PCR) of the nucleic acid (RNA) are the standard methods for diagnosing SARS-CoV-2 infection,<sup>11-13</sup> but they might have some disparities in their ability to accurately detect the viral target gene(s), especially in specimens with very-low viral titers or with emerging variants.<sup>10-14</sup> Of note, diagnostic trials have been conducted extensively prior to the EUA, but the routine use of these assays in real-life remains poorly documented. Furthermore, the lack of evidence on the diagnostic concordance between existing systems limits the potentials to ensure assay inter-operability for the further validation in routine clinical diagnosis. In several sub-Saharan African countries, *Abbott* and *DaAn gene* molecular systems have been widely used in response to COVID-19 molecular detection.<sup>7,8</sup> Thus, evaluating the diagnostic agreement would be of paramount importance in the routine COVID-19 diagnostic landscape in these settings, in an accurate detection of suspect cases for adequate clinical management, and in an optimal public health control measures through the identification and tracking of potential transmitters at community-level. We herein evaluated the diagnos-

tic concordance of *Abbott* and *DaAn gene* rRT-PCR on both standard panels and clinical specimens.

## Materials and Methods

### Study design and population

Within the frame of the EDCTP PERFECT-Study (Performance Evaluation of COVID-19 Tests), a comparative study was conducted on 287 samples [273 nasopharyngeal clinical swabs and 14 quality control (CQ) panels] enrolled from February through May 2021 at the Chantal BIYA International Reference Center (CIRCB) for research on HIV/AIDS prevention and management. Study population was therefore made up of travelers, symptomatic patients, and contact cases; while panels for external quality control assessment were provided by the WHO country office and the African Society for Laboratory Medicine (ASLM) through a partnership with the Africa CDC.

Nasopharyngeal swabs were collected from consenting individuals and stored in tubes containing a Viral Transport Media (VTM) appropriate for COVID-19 molecular testing (Sansure Biotech Inc, <https://www.appletonwoods.co.uk>).

For each participant, the nasopharyngeal sample was then divided into two aliquots for a parallel laboratory processing on *Abbott* and *DaAn gene* rRT-PCR respectively. Socio-demographic and basic clinical data were collected using a standard datasheet for COVID-19 testing.

### Description of the study site

CIRCB is a national reference laboratory for the molecular diagnosis of COVID-19 under the Ministry of Public Health of Cameroon. To ensure reliability in COVID-19 testing, CIRCB participates in external quality control programs for proficiency testing with the WHO and the ASLM. A total of 41,716 samples have been tested so far. CIRCB also has a sequencing platform dedicated to HIV genotyping and activities related to the COVID-19 genomic surveillance platform in Cameroon.

### Laboratory procedures

#### *Sample collection and processing*

All clinical samples were collected from individuals tested for COVID-19 diagnosis in Yaoundé. Briefly, nasopharyngeal swabs were collected, by trained personnel, in a 1mL tube containing viral transport medium as per manufacturer's instructions and under universal biosafety measures.

After collection, the samples were stored between 4°C and 8°C for 2-5 hours, and referred for laboratory processing.

#### *Nucleic acid extraction, amplification and detection with Abbott platform*

For *Abbott* rRT-PCR assay for SARS-CoV-2 detection a 1000µL aliquot of each inactivated sample (500µL nasopharyngeal swab + 500µL of DNase-RNase free water heated at 70°C for 10min) was loaded into *Abbott* m2000sp instrument, combined with the *Abbott* SARS-CoV-2 master mix containing an internal RNA control, primers, and probes targeting both an RNA dependent RNA polymerase (RdRp) gene, specific for SARS-CoV-2 as well as the conserved structural protein nucleocapsid (N) gene ([www.fda.gov/media/136258/download](http://www.fda.gov/media/136258/download)).

Amplification was performed using thermocycler m2000rt after automated extraction and sample preparation using *Abbott* m2000sp instrument. At the end of amplification process, negative results were rendered as "target not detected" (no amplification was observed after 37 cycle numbers); meanwhile positive results were rendered as "target detected" with a given number representing the Cycle Number (CN) at which the detection phase was initiated. This CN value was inversely proportional to the viral load of the patient. According to the manufacturer, detection sensitivity for this assay is 100 copies/mL.

#### *Nucleic acid extraction, amplification and detection with DaAn gene platform*

For *DaAn gene* rRT-PCR assay, viral RNA was manually extracted from 140µL nasopharyngeal swab using the QIAamp Viral RNA Mini Kit (Qiagen Inc, Valencia, CA, USA) as per manufacturer's instructions. Amplification was performed using the *DaAn gene* RT-PCR kit ([www.daan-gene.com](http://www.daan-gene.com)) on the Quant Studio 5 (ThermoFisher). The protocol used probes targeting the open reading frame (ORF1ab) gene and the nucleocapsid (N) protein gene, with a lower limit of detection of 500 copies/mL and an amplification reaction of 45cycles. As per the manufacturer's instructions, each sample with a Cycle Threshold (CT) value <40 was considered positive, while a CT value >40 or "undetermined" (no amplification after 45 cycles) was considered negative; according to national guidelines in Cameroon, we also evaluated the positivity of *DaAn gene* at a CT value<37; considering the threshold for SARS-CoV-2 transmissibility, we further evaluated the positivity of *DaAn gene* at a CT value<35.

### Statistical analyses

Statistical analyses were performed using Graph-Pad software version 6; correlation analyses were done using the Spearman's correlation test; the concordance in diagnosis was evaluated following the Cohen's kappa (k) value, and results were interpreted according to the criteria proposed by Landis & Koch: k=0.01-0.20 (poor concordance), k=0.21-0.40 (fair concordance), k=0.41-0.60 (moderate concordance), k=0.61-0.80 (strong concordance), and k=0.81-1.00 (almost perfect concordance).<sup>15</sup> The diagnostic concordance was then compared according to different CT-values (<40 as per the manufacturer's instructions; <37 and <35 as aforementioned) of *DaAn gene* assay. All p-value<0.05 were considered statistically significant, with 95% confidence interval.

### Ethics

The study has obtained ethical approval from the Cameroon National Ethics Committee for Human Health Research (reference N°01/143/CNERSH/SP). Per the Helsinki's declaration and the national regulations, informed consent was obtained from all participants; confidentiality was ensured through de-identification by the use of a unique identifier for each participant and the storage of data in a password-protected computer.

## Results

### Sociodemographic and clinical features of the study participants

Out of the 273 participants enrolled in this study 57.88% (158/273) were males and 42.12% (115/273) females; the median (IQR) age was 36 (26–46) years; and about 4% (10/273) of the study population reported a COVID-19 related symptom.

### Positive and negative concordance between *Abbott* and *DaAn gene* assays according to the cycle thresholds for SARS-CoV-2 molecular diagnosis

According to the various CT thresholds in SARS-CoV-2 molecular diagnosis of the *DaAn gene* assay (<35, <37, <40 as per the manufacturer's instructions), a declining concordance was observed between *Abbott* and *DaAn gene* for CTs values <35 (k=0.84), <37 (k=0.82), <40 (k=0.78), with similar concordance at CT-values of 35 and 37 (Table 1).

Considering the overall concordance with each CT-value, the CT<37 was consid-

ered as the suitable threshold for positivity and has been used for subsequent analyses in this study.

### Concordance between *Abbott* and *DaAn gene* protocols

The *Abbott* real-time SARS-CoV-2 assay showed a positivity rate of 29.96% [95%CI: 25.0–35.5%] (86/287) versus 37.63% [95%CI: 32.2–43.4%] (108/287) for the *DaAn gene* assay. The overall percentage agreement between these two assays was 82.57% (237/287), with k=0.82 (95% CI: 0.78-0.86), indicating an excellent concordance between the two diagnostic protocols.

The positive and negative concordance were 66.67% (72/108) and 92.18% (165/179) respectively (Table 2).

### Concordance between *Abbott* and *DaAn gene* according to the presence or absence of COVID-19 related symptoms

The positivity rate of the *Abbott* and *DaAn gene* protocols in symptomatic patients were respectively 80% and 100%; and respectively 26.23% and 33.84% asymptomatic patients (Figure 1). Looking at the confidence intervals, no significant difference was found between *Abbott* and

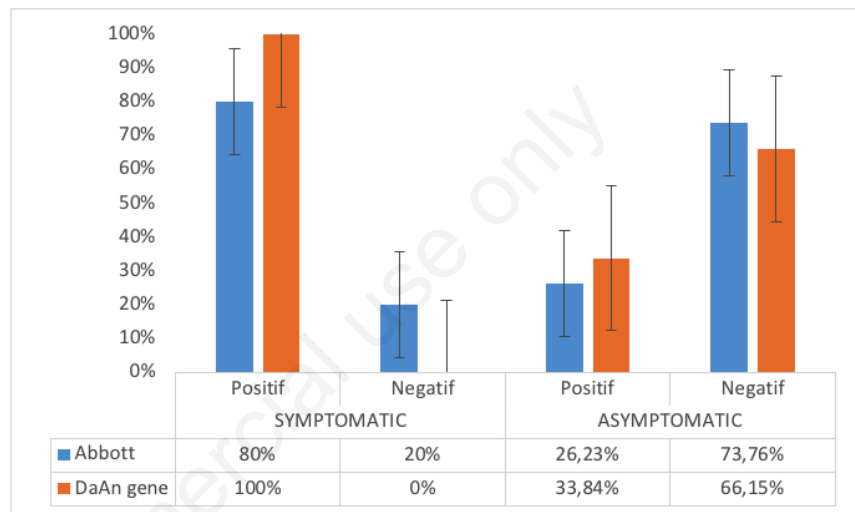


Figure 1. Positive and negative rates between *Abbott* and *DaAn gene* using in asymptomatic and symptomatic patients.

Table 1. Positive and negative concordance between *Abbott* and *DaAn gene* at CT/CN: 35, 37 and 40.

	Percentage (%), Kappa
Concordance (CT<35)	
Positive concordance (67/93)	72.04
Negative concordance (175/194)	90.20 k: 0.84 (95% CI: 0.795-0.883)
Concordance (CT<37)	
Positive concordance (72/108)	66.66
Negative concordance (165/179)	92.18 k: 0.82 (95% CI: 0.777-0.863)
Concordance CT<40	
Positive concordance (73/122)	59.83
Negative concordance (152/165)	92.12 k: 0.78 (95% CI: 0.735-0.820)
Concordance (EQC)	
Positive concordance (9/9)	100
Negative concordance (5/5)	100

Table 2. General Concordance between *Abbott* and *DaAn gene*.

<i>DaAn gene</i>	Positive	<i>Abbott</i> Negative	Total	Kappa (k, 95% CI)
Positive	72	36	108	0.82 (0.78 – 0.86)
Negative	14	165	179	
Total	86	201	287	

*DaAn* gene protocols according to whether or not the patient presented with a Covid-19 related symptom. In our study population, the concordance between *Abbott* and *DaAn* gene in symptomatic and asymptomatic patients were  $k=0.80$  (95% CI: 0.80 - 0.80) and  $k=0.82$  (95% CI: 0.77 - 0.86) respectively.

**Positive concordance between *Abbott* and *DaAn* gene assays according to viral load estimates**

Regarding the viral load estimates, the lower the CT/CN values, the higher the concordance between these two tests. Specifically, 100% positive agreement was found between the two tests and the mean gene when CT was <20, 93.10% when the mean CT was 88.88% between [20-30], 66.67% with a mean CT of 60.61% between [30-35], 48.27% with a mean CT of 29.17% between [35-37], and 12% with a mean CT of 10% between [37-40] (see details in Table 3).

**Correlation between *Abbott* CN and *DaAn* gene CT values**

We obtained strong positive correlations between *Abbott* CN and all *DaAn* gene viral targets' CTs; with ORF1ab gene, we found  $r=0.76$ ,  $p<0.0001$  and 95% Confidence Intervals (CI) [0.74–0.89]; with N gene, we found  $r=0.80$ ,  $p<0.0001$  and 95% CI [0.72–0.88], we combined both viral targets' CT values under a unique number and we labelled as “Mean” and obtained a good correlation ( $r=0.81$ ;  $p<0.0001$  and 95% CI [0.77–0.90]; Figure

2). Additionally, we found a good correlation ( $r=0.96$   $p<0.001$ ) with N gene using positive samples of EQC panels (Figure 3).

**CT and CN variation constant using the curve equation from spearman correlation**

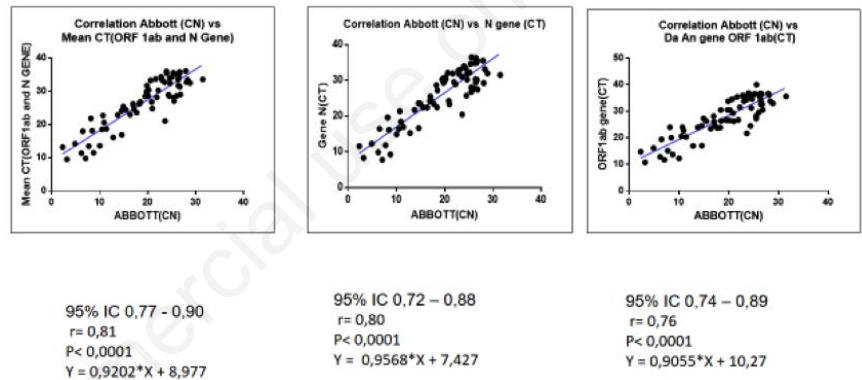
Using the curve equation from spearman correlations, we obtained different constants in relation with *Abbott* CN and *DaAn* gene viral targets (N, ORF 1ab, Mean and EQC controls see Table 4 and Figure 4). These constants enabled the extrapolation of our data in order to estimate interchangeability of the results between the two protocols among positive swabs according to the gene of interest.

*DaAn* gene values was determined based on a given *Abbott* values and vice versa. The difference in CT and CN

between the two tests was on average  $6.75\pm 0.3$ .

**Discussion**

In the frame of ongoing COVID-19 pandemic,<sup>16</sup> SARS-CoV-2 diagnosis is critical for the detection of infected individuals, contact-tracing and mitigating the viral transmission.<sup>12</sup> In this context, identifying the concordance between commonly used assays such as automated (*Abbott*) and manual (*DaAn* gene) rRT-PCR assays has a public health significance. In this study, participants were relatively young (median age of 36 years), reflecting the demography of most African countries. According to gender distribution, females were less represented than males, suggesting a possible



**Figure 2. Correlation between *Abbott* CN and the *DaAn* gene CT values according to gene of interest.**

**Table 3. Positive concordance between *Abbott* and *DaAn* gene assays according to viral concentration with (ORF1ab, N and mean) genes.**

CT value	gene ORF1ab			gene N			Mean (ORF1ab, N)gene		
	<i>DaAn</i> gene, (n)	<i>Abbott</i> (n)	Concordance (%)	<i>DaAn</i> gene, (n)	<i>Abbott</i> (n)	Concordance (%)	<i>DaAn</i> gene, (n)	<i>Abbott</i> (n)	Concordance (%)
CT <20	12	12	100	16	16	100	14	14	100
CT [20-30]	29	27	93.10	36	27	75	33	29	88.88
CT [30-35]	24	16	66.67	41	24	58.54	33	20	60.61
CT [35-37]	29	14	48.27	17	5	29.41	24	7	29.17
CT [37-40]	25	3	12	12	1	8.33	30	3	10

**Table 4. Variation constants obtained through the curve equation from spearman correlation.**

	Equation	Variation Constant
<i>Abbott</i> vs. Mean (N & ORF 1) gene	$Y = 0,9202 * X + 8,977$	8,977
<i>Abbott</i> vs. N gene	$Y = 0,9568 * X + 7,427$	7,427
<i>Abbott</i> vs. ORF 1ab gene	$Y = 0,9055 * X + 10,27$	10,27
<i>Abbott</i> vs. Mean (N & ORF 1) gene (EQC)	$Y = 0,9097 * X + 10,95$	10,95
<i>Abbott</i> vs. N gene (EQC)	$Y = 0,9889 * X + 10,23$	10,23
<i>Abbott</i> vs. ORF 1ab gene (EQC)	$Y = 0,8300 * X + 11,69$	11,69

male vulnerability to COVID-19.<sup>17-19</sup>

According to the clinical status, only few participants were symptomatic patients, which could be partly explained by the youthfulness of our study population, but this also underlines the important circulation of asymptomatic cases (silent spreader of the disease) in the general population as reported by other authors.<sup>20,21</sup>

We found an excellent concordance between the *Abbott* and *DaAn gene* assays using the Cohen's kappa coefficient ( $k=0.82$ ), suggesting a good diagnostic agreement between the two platforms. Overall, this study revealed that *DaAn gene* is more sensitive in detecting SARS-CoV-2 infection in symptomatic and asymptomatic patients. Such considerations as the method of extraction (manual or automated), as reported by several authors,<sup>12,13</sup> may help to explain these results. The slight discrepancy observed between the two tests could be

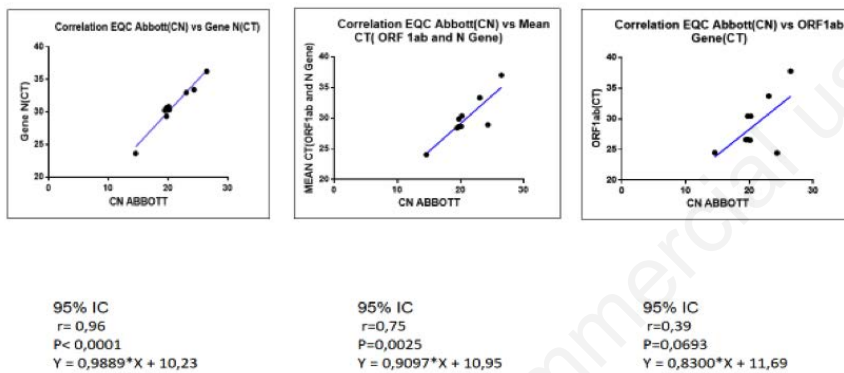
related to the quality of the PCR (component, target) and the detection limit, which may influence the performance of one test over the other.<sup>22,23</sup>

Additionally, the RdRp gene targeted by *Abbott* is produced by the ORF1b which is more exposed than the N gene to host immunity and thus more susceptible to mutations.<sup>8,24</sup>

Taking into consideration the  $CT < 35$ , as well as the locally adopted thresholds ( $CT < 37$ ) and the manufacturer's one ( $CT < 40$ ), the concordance was similar at  $CTs < 35, < 37$ , reinforcing the accuracy of the local guidelines in COVID-19 diagnosis. Besides, our results show strong positive correlations between *Abbott* CN and all *DaAn gene* viral targets' CTs (N gene, ORF1ab and Mean) when considering only positive results. The CT value obtained with the Mean value and N gene had a better correlation with *Abbott* CN than ORF1ab, prob-

ably because assay performance varies between PCR targets as reported previously.<sup>23</sup> Also, several authors reported good stability and high production of N gene among infected cells,<sup>25,26</sup> which may also justify the better correlation of their CT values with *Abbott* CN as compared to that of ORF1ab gene.

Interestingly, we obtained an excellent correlation using the EQC samples panels ( $r=0.96$ ) on N gene, suggesting a better stability and reliability of synthetic samples compared to biological samples throughout the extraction and amplification processes in COVID-19 testing. In view of that, quality control gives confidence in diagnosis interpretation. These results, globally, suggest the interchangeability of these two assays regarding positive nasopharyngeal swabs. Moreover, extrapolation of our data using variation constants obtained from correlation equations in this study, according to gene of interest, enlighten more the consideration of a possible algorithm for the interpretation of the results among positive specimens in our country; especially as these two assays are the most used locally. In perspective, assessing the cost-effectiveness of the two assays, with reference to a gold standard (digital droplet PCR, etc) would be of great relevance.



**Figure 3.** Correlation between *Abbott* CN and the *DaAn gene* CT values according to gene of interest among EQC.

### Conclusions

The excellent agreement (>80%) between the *Abbott* and *DaAn gene* rRT-PCR platforms supports inter-operability between the two assays in the diagnosis of SARS-CoV-2 in tropical settings like Cameroon. The acceptable rate of discordance (<20%) often occurs at low-VL (threshold with limited risk of viral transmission), thus supporting the efficiency of these assays in the global surveillance and control of COVID-19 pandemic.

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4,1			4,2			
Fixed <i>Abbott</i> (CN)	Genes	Corresponding <i>DaAn gene</i> (CT)	Genes	Fixed <i>DaAn gene</i> (CT)	Corresponding <i>Abbott</i> (CN)	
15	Mean (N,ORF)	22,78	Mean(N,ORF)	15	6,54	
	N gene	21,77		20	11,97	
	ORF1ab gene	23,85		25	17,41	
20	Mean (N,ORF)	27,38		30	22,84	
	N gene	26,56		15	7,91	
	ORF1ab gene	28,38		20	13,14	
25	Mean (N,ORF)	31,98	N gene	25	18,36	
	N gene	31,34		30	23,59	
	ORF1ab gene	32,9		15	5,22	
30	Mean (N,ORF)	36,58		ORF1 ab gene	20	10,74
	N gene	36,13			25	16,26
	ORF1ab gene	37,43			30	21,78

**Figure 4.** Estimated CT and CN obtained through the curve equation from spearman correlation. Using the equation from the spearman correlation shown in Table 3, we determined *DaAn gene* values based on given *Abbott* values and vice versa. With  $Y = DaAn\ gene\ CT$  and  $X = Abbott\ CN$ ; with  $Y=AX + B$  and  $X=(Y-B)/A$ . (4.1) represents corresponding *DaAn gene* CT values obtained through fixed *Abbott* CN; (4.2) represents corresponding *Abbott* CN values obtained through fixed *DaAn gene* CT.

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