Inter-Site Validations of the Pixel-Wise Method for Cardiac T2* Analysis in Transfusion-Dependent Thai Thalassemia Patients

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Objective: *To compare inter-site observer variability of the Pixel-Wise (PW) method for cardiac T2* analysis in thalassemia patients using the mono-exponential with a constant fitting (offset) model and to compare the cross-model variability of the offset model to the mono-exponential (typical) model.*
Material and Method: *Eighty-eight cardiac T2* measurements were performed on 72 Thalassemia major patients. Both bright- and black-blood techniques were acquired and analyzed at both the reference (REF) and local (LOC) sites using the PW method by defined region of interest on the whole (at the REF site) and partial (at the LOC site) septum. The offset model was analyzed at the reference site while both the offset and typical models were performed at the local site. The inter-site variability of the T2* values were analyzed by independent observers blinded to the results.*
Results: *The T2* values from the REF-offset, LOC-offset and LOC-typical methods were statistically comparable on both scanning techniques. The inter-site variations of the offset model were about 5.2% and 4.4% on the bright- and black-blood techniques, respectively, which was about 1.7% higher than from the intra-site, but was still in a reasonable range compared to the conventional method of around 5.4%. The cross-model comparisons presented with 0.4 ms of bias and variation of about 6.9% and 4.7%, respectively, which is about 1.4% higher than from the intra-site.*
Conclusion: *The observer variability on the PW method using the offset or typical model provided equivalent coefficient of variation on both scanning techniques, which was also comparable to the previous reports. The inter-site variability of the offset and cross models was also in a reasonable range, being less than 2% higher than the intra-site with bias of about 0.4 ms.*

Keywords: *CMR, Myocardial T2* analysis, Pixel-Wise method, Thalassemia; Iron overload; The offset model, The mono-exponential model*

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Cardiac T2* measurements are a fundamental tool for assessing myocardial iron overload in Thalassemia patients[1-3]. Cardiac T2* is reciprocally related to myocardial iron concentration and has been validated in animal and post mortem studies[4-6]. More importantly, cardiac T2* predicts the risk of cardiac events[7], which remain the major cause of death in patients with transfusion-dependent thalassemia. As a result, cardiac T2* measurement has become the defacto standard for monitoring iron overload in clinical trials of the iron chelation therapy and also in clinical care of the long-term transfusion patients[8-10].

Technically, the measurement can be separated into Magnetic Resonance (MR) acquisition

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and post-processing-analysis processes. Robust image quality can be obtained using multi-echo gradient echo imaging in a single breath-hold time[11,12], utilizing either bright- or black-blood contrast. The bright-blood technique[11] is an earlier proven technique that is still employed with some previous MR scanner platforms. Black-blood techniques[12], on the other hand, are generally considered superior because flow and motion artifact suppression improves edge detection and lowers T2* variability.

Greater heterogeneity is found in post-processing analysis of the T2* measurement, with commercially available software such as CMR-Tools (Cardiovascular Imaging Solution, London, UK), CMR42 (Circle Cardiovascular Imaging, Calgary, CA), and, FuncTools (GE Medical Systems, Milwaukee, WI), as well as custom-written research software[13-15]. The analysis can be conceptually divided into two groups: the Region-Based (RB)[16] and Pixel-Wise (PW)[10,13,17] methods. The RB method begins with the averaging of each echo signal using a defined region of interest (ROI) and then fitting it to the exponential models. The PW method, on the other hand, fits the decay signal point by point and then averages the R2* (the reciprocal of T2*) values from the defined ROI. The RB method is a fast and robust technique[18,19] but cannot provide the distribution pattern of T2* values in the myocardium. The PW method, in contrast, can present such the profile but is more computationally intensive. For the current single-breath-hold acquisition technique, both RB and PW methods are technically feasible and produce results that are in close agreement[10,20], excepted for the susceptibility-artifact regions.

There continues to be ongoing debate concerning the choice of the exponential fitting models. There are currently three accepted models for the RB method: the mono-exponential (typical), mono-exponential with constant (offset) and mono-exponential with truncation (truncation)[5,20,21]. Recent work[5] suggests employing the typical and truncation models for the black- and bright-blood techniques, respectively. The suggestions, however, are based on the RB method. The choices of fitting models for the PW method, on the other hand, are still open for further investigation. Thus there were two objectives in the present study. First, the authors investigated the inter-observer variability of the reference site (Children's Hospital Los Angeles, CA, USA) to our institute (Siriraj Hospital, Bangkok, Thailand) using the offset model on the PW method. The second objective was to compare the offset model from the reference site to the authors MPS-PW, which the authors have previously shown yields lower intra-observer variability as compared to the conventional RB or typical PW methods[22].

Material and Method

Study Group

Eighty-eight Cardiac T2* measurements were performed on 72 Thalassemia major patients (32 males and 40 females, age 18.0 $\pm$ 6.9 years) who received regular transfusion and iron chelation therapy and were aged $\geq$ 10 years with serum ferritin levels > 1,000 ng/ml. Patients with a contraindication to MR, including pacemakers, claustrophobia, and inability to comply with the instructions were excluded from the study. Approval was obtained from the ethics committee of Faculty of Medicine Siriraj Hospital.

MRI and Interpretation

The images were acquired on a 1.5T Philips Achieva XR Quasar Dual Gradient system using a five-element cardiac phased-array coil. Each patient was scanned using both the optimized bright-blood[23,24] and the original black-blood[12] sequences. In summary, each technique is a cardiac-gated multi-echo fast gradient sequence acquired within a single breath-hold time. The black-blood technique employs an additional double inversion recovery pre-pulse to null blood signal in the heart chamber. A single midventricular short axis slice was acquired by both techniques with a slice thickness of 6 and 10 mm for the bright- and black-blood techniques, respectively. Imaging parameters for the bright-blood technique were a repetition time (TR) of 19 ms, eight echo times (2.2-17.6 ms with 2.2 ms increments), a matrix of 192 x 256 and a field of view (FOV) of 40 cm, which yielded a voxel size of 2.1 x 1.6 x 6 mm^3. For the black-blood technique, imaging parameters were a TR of 19 ms, eight echo times (2.6-16.74 ms with 2.02 ms steps), a matrix of 128 x 256 and a FOV of 40 cm, which yielded a voxel size of 3.1 x 1.6 x 10 mm^3 while the inversion time (TI) was set to suppress the blood signal. The bright-blood images were acquired during the late systole and diastole for the black-blood images.

The acquired images were analyzed locally and also transferred in DICOM format to process at the reference site. All T2* analyses were performed independently at both sites using individually custom-written software developed in MATLAB (The MathWork, Natick, MA)[22,24]. All image data were fit to

the offset model at the reference site (REF-offset) and fit to the offset (LOC-offset) and mono-exponential (LOC-typical) models at the local site. ROIs had been defined, manually, from the whole interventricular septum (WS) at the reference site, as in the conventional method and from the partial interventricular septum (PS) region using the prior knowledge of a T2* map to avoid susceptibility artifact and partial volume effect at the edge of septum, as suggested in previous studies[10,17,22]. MR technologists with more than ten years experience in functional cardiac MR analysis independently performed the analyses. The T2* results of all models in this study were reported by using both their mean and median values. The result from LOC-typical method reported by its median is, therefore, equivalent to the MPS-PW method.

Statistical analysis

All statistical analyses were evaluated using MATLAB Statistics Toolbox. Bland-Altman plot was employed to analyze the agreement between two different T2* data sets. The coefficient of variation (CV) is utilized as the quantitative analysis of the closeness of the agreement. The CV was calculated from the standard deviation of the differences between two data sets, then divided by their mean and presented as percentage. A paired Student's t-test was selected to evaluate the difference between the two data. A p-value less than 0.05 were considered to be significant in this study.

Results

An example of cardiac images and their corresponding T2* maps of a thalassemia patient, in which T2* value in myocardium was 21 ms (marginally overload), are depicted in Fig. 1. The top (Fig. 1A-C) and bottom (Fig. 1D-F) rows are acquired images and analyze maps from the bright- and black-blood techniques, respectively. The first and fourth echo images and their T2* maps, overlaid with ROIs from PS regions (the blue lines), which excluded the high T2* values (the light green area) around the edge of myocardium and the low T2* values (the red area) in the inferior septum, are shown on each row.

The mean T2* values reported by its median from the REF-offset, LOC-offset and LOC-typical methods were 30.4 $\pm$ 13.8, 30.1 $\pm$ 13.8 and 30.0 $\pm$ 13.5 ms ($p = 0.8775$ and 0.8345, respectively) for the bright-blood technique, as well as 28.8 $\pm$ 12.0, 28.9 $\pm$ 12.3 and 28.4 $\pm$ 12.0 ms ($p = 0.9746$ and 0.8379, respectively) for the black-blood technique, respectively. The mean T2*

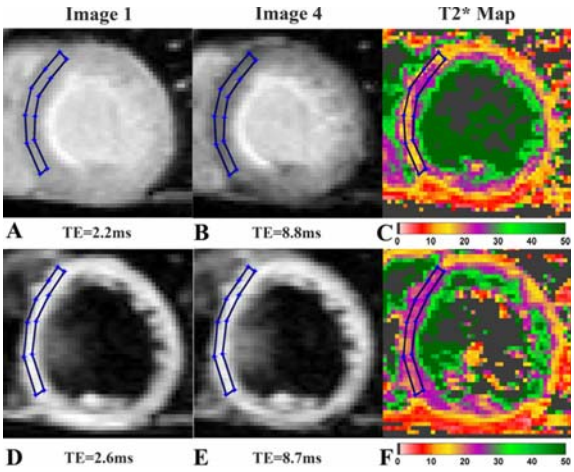

Fig. 1 Cardiac images and T2* maps overlaid with ROIs of the PS region (the blue lines). Bright- (A and B) and Black- (D and E) Blood cardiac images and corresponding T2* maps (C and F) are displayed at the top and bottom rows, respectively. The color-map scale bar for T2* map is shown at the bottom in units of milliseconds

values reported by its mean also had no statistical difference. The T2* analysis from the offset model using the PW method at the reference site (REF-offset) was, hence, of statistically insignificant difference as compared to that of the same (LOC-offset) or typical (LOC-typical) model at the local site.

Table 1 presents bias, 95% confidence interval (CI) and the CV of the intra- and inter-site variations from the offset model while the results from the typical and cross-model (offset vs. typical) comparisons are demonstrated on Table 2. The upper part of both tables shows the results using the mean report while the median one is on the lower part. As shown on both tables, the variations from the median report were lower than the mean report for all comparisons. The presentation in this study, hence, is focused only on the variations from the median report.

Fig. 2 demonstrates the Bland-Altman plots of the intra- and inter-site observer variations of the T2* values from both bright- and black-blood techniques using the offset model. The corresponding bias, 95% CI and the CV of this figure are presented at the lower part of Table 1. As demonstrated in the figure and table, the observer variability of the offset model on both scanning techniques had low bias (< 0.5 ms) and reasonable variations (< 6%). The intra-site variations of the offset model were about 3.6% and 2.7% on the bright- and black-blood techniques,

Table 1. Bias, 95% CI, and CV from comparison of T2* values from reference (REF) and local (LOC) sites using offset model on the bright- and black-blood techniques reported by its mean and median

	Bright-Blood Technique		Black-Blood Technique	
	Bias [95% CI] (ms)	CV (%)	Bias [95% CI] (ms)	CV (%)
MEAN-REPORT				
LOC-offset vs. LOC-offset	-0.12 [-2.99 to 2.76]	4.16	-0.03 [-1.79 to 1.72]	3.09
REF-offset vs. LOC-offset	0.64 [-2.69 to 3.98]	6.10	0.43 [-2.18 to 3.05]	4.92
MEDIAN-REPORT				
LOC-offset vs. LOC-offset	-0.13 [-2.49 to 2.21]	3.58	-0.04 [-1.47 to 1.39]	2.67
REF-offset vs. LOC-offset	0.43 [-2.90 to 3.54]	5.19	-0.06 [-2.37 to 2.26]	4.43

Table 2. Bias, 95% CI, and CV from comparison of T2* values from the reference (REF) and local (LOC) sites using offset and typical models on the bright- and black-blood techniques reported by its mean and median

	Bright-Blood Technique		Black-Blood Technique	
	Bias [95% CI] (ms)	CV (%)	Bias [95% CI] (ms)	CV (%)
MEAN-REPORT				
LOC-typical vs. LOC-typical	-0.12 [-2.87 to 2.63]	4.22	-0.08 [-1.75 to 1.59]	3.06
LOC-offset vs. LOC-typical	-0.07 [-3.08 to 2.94]	5.91	0.33 [-1.63 to 2.29]	3.64
REF-offset vs. LOC-typical	0.69 [-2.64 to 4.01]	5.95	0.80 [-1.81 to 3.40]	4.80
MEDIAN-REPORT				
LOC-typical vs. LOC-typical	-0.10 [-2.38 to 2.17]	3.53	-0.07 [-1.50 to 1.37]	2.57
LOC-offset vs. LOC-typical	0.21 [-2.38 to 2.81]	5.48	0.39 [-1.43 to 2.20]	3.42
REF-offset vs. LOC-typical	0.45 [-3.64 to 4.53]	6.88	0.44 [-1.86 to 2.74]	4.69

respectively. As compared to the intra-site variations, the inter-site variations had higher CV by about 1.6% and 1.7% on the bright-blood technique (5.2%) and black-blood technique (4.4%), respectively. The inter-site variation of the bright-blood technique, however, had higher bias (0.43 vs. 0.03 ms) as compared to the intra-site. The inter-site variations of the offset model, hence, were less than 2% higher than the intra-site variation on both scanning techniques, but with some higher bias on the bright-blood technique.

The Bland-Altman plots of the intra-site variations of the typical model on both scanning techniques are depicted in the first row of Fig. 3 and their bias, CI and CV are presented in Table 2. The intra-site variations of the typical model were about equivalent to the offset model for both the bright-blood technique (3.5% vs. 3.6%, $p = 0.8401$) and black-blood technique (2.6% vs. 2.7%, $p = 0.7733$). The intra-site variations of the offset and typical models were, hence, comparable on both scanning techniques.

The Bland-Altman plots of the intra- and inter-site variations of the cross model (offset vs. typical) comparisons are depicted on the second and third rows of Fig. 3 while the corresponding bias, 95% CI and the CV are presented in Table 2. The cross-model comparisons also had low bias (< 0.5 ms) and reasonable variations (< 7%) for both scanning techniques, but present with some bias (0.4 ms) in the inter-site variations as compared to the intra-site. The intra-site CVs of the cross model were about 5.5% and 3.4% for the bright- and black-blood techniques, respectively, which is about 2.0% and 0.6% higher than the CV from the intra-site offset model. Moreover, the inter-site variations of the cross model from the bright and black-blood techniques were about 1.4% and 1.3% higher than from the intra-site variations.

In summary, the intra-site variability of the offset and typical models was equivalent; there were both low bias and reasonable variations from both scanning techniques. The variations from the black-blood technique were lower than those from the bright-blood technique for all comparisons. The inter-site CV

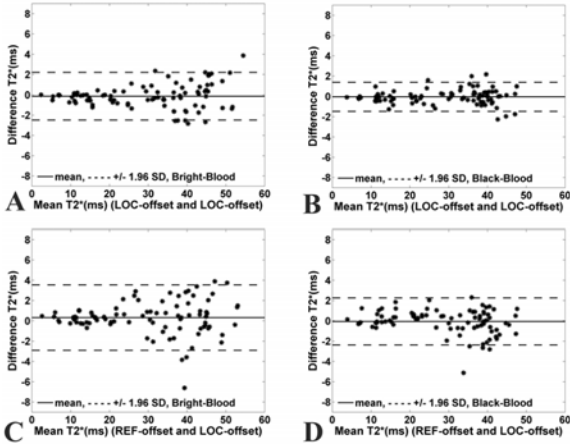

Fig. 2 Bland-Altman plots comparing myocardial T2* values of the intra- (the top row) and inter- (the bottom row) site observer variations of the reference (REF) and local (LOC) sites using the offset model reported by its median (the lower part of Table 1). The results from the bright- and black-blood techniques are displayed at the left and right columns, respectively

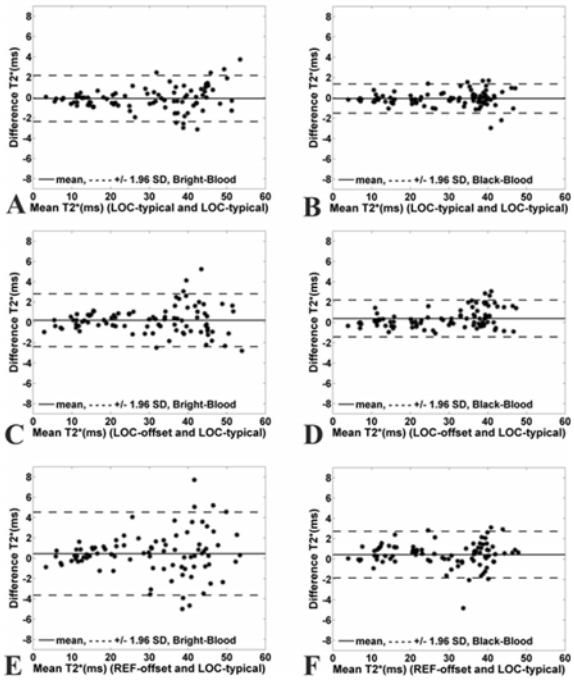

Fig. 3 Bland-Altman plots comparing myocardial T2* values of the intra- (the first and second rows) and inter- (the third row) site variations from the reference (REF) and local (LOC) site using offset and typical models reported by its median (the lower part of Table 2). The results from the bright- and black-blood techniques are displayed at the left and right columns, respectively

of the offset model from both scanning techniques was about 1.7% higher than from the intra-site and about 1.4% higher for the cross-model comparisons.

Discussion

The analysis of cardiac T2* measurement using the conventional RB method is well established for its variability and reproducibility studies[19,25,26], while the PW method has recently gained more acceptance, as it presents fewer technical difficulties when applied to the current single breath-hold acquisition[10,13,17]. The authors had demonstrated that the MPS-PW method which employed the typical model provided substantially lower observer variability as compared to the RB method for both scanning techniques[22]. The intra-site CV of both offset and typical models on the PW method from both scanning techniques, found in the present study, was equivalent and the inter-site CV of the offset and cross-model comparisons were within reasonable ranges as compared to the same comparison from the intra-site.

In the present study, for all comparisons, T2* measurements, which were reported by using their median, gave lower CV than using the mean and the results from the black-blood technique had lower CV than from the bright-blood technique. Reported by using its median can lower the CV and could be due to the reduction of the T2* outliers (abnormal) effect generated by the motion artifacts[22]. However, the different CVs on both scanning techniques were not only due to the artifacts, but also to differences in the Signal to Noise Ratio (SNR) and trigger delay. The imaging parameters for both acquisition sequences employed in this study were defined from the previous published reports[12,23]. The bright-blood technique had SNR lower by about 41% as compared to the black-blood technique, due to the voxel size and the bright-blood images were acquired during the late systolic phase (to reduce motion artifact) while the black-blood images were scanned during the diastole phase (to optimize the suppression of blood signal). Such differences may contribute to the lower CV in the black-blood technique, which also had been noted previously in reports[22,27].

The intra-site observer variability of the offset and typical model on the PW method provided equivalent CV on both scanning techniques and was also comparable to the previous published results. The intra-site CV of both models was about 3.6% and

2.7% for the bright- and black-blood techniques, respectively, which is similar to the previous report of 3.5% and 2.4% from the MPS-PW method[22] and of 4.5% and 2.5% from the recent conventional RB method[27]. Both models, therefore, can provide the similar reproducibility for the PW method. Furthermore, the inter-site CV of the offset model from the bright- and black-blood techniques was about 5.2% and 4.4%, respectively, which was about 1.7% higher than from the intra-site variations. Such differences should result from the inter-site observer variations as well as from the different ROIs defined by each site (WS vs. PS). Therefore, the higher bias (0.4 ms) found only in the bright-blood technique could be due to a difference in ROI. The inter-site variation of the offset model, nevertheless, was still in a reasonable range when compared to the reproducibility from the RB method of around 5.4%[25].

The intra-site cross-model variation from the bright- and black-blood techniques was also in a reasonable range with CV of 1.9% (5.5% vs. 3.6%) and 0.7% (3.4% vs. 2.7%), respectively, higher than that of the offset model. Moreover, the inter-site variations of the cross mode were about 6.9% and 4.7%, which are about 1.4% higher than the intra-site variations.

Further investigation should focus on the inter-site variability of the typical and offset models, specifically for patients with heavy cardiac iron loading (T2* < 5 ms), of which there were too few in this study; this would give more insight of such differences and would be of clinical interest.

Conclusion

The intra-site observer variability on the PW method using the offset or typical model provided equivalent CV on both the bright- and black-blood techniques that was also comparable to the previous reports. The inter-site variability of the offset and cross models was also within a reasonable range, being less than 2% higher than the intra-site with a bias of about 0.4 ms.

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Potential conflicts of interst

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ตรวจสอบความสมเหตุสมผลระหว่างสถาบันสำหรับการวิเคราะห์ค่า T2* ของหัวใจโดยวิธีแบบจุดภาพในผู้ป่วยธาลัสซีเมียในไทยที่ต้องได้รับการถ่ายเลือด

ไพรัช สายวิรุณพร, วิปร วิประกษิต, อดิศักดิ์ มณีไสย, นพดล ศิริธนารัตนกุล, บุญชู พงศ์ธนากุล, John C Wood, รุ่งโรจน์ กฤตยพงษ์

วัตถุประสงค์: *เพื่อเปรียบเทียบความเปลี่ยนแปลงจากผู้สังเกตการณ์ระหว่างสถาบันในการวิเคราะห์ค่า T2* ของหัวใจด้วยวิธีแบบจุดภาพในผู้ป่วยธาลัสซีเมียโดยใช้แบบจำลองแบบ mono-exponential with a constant (offset) และเพื่อเปรียบเทียบความเปลี่ยนแปลงของแบบจำลองดังกล่าวกับแบบ mono-exponential (typical)*

วัสดุและวิธีการ: *ได้ทำการวัดค่า T2* ของหัวใจจำนวน 88 ครั้ง จากผู้ป่วยธาลัสซีเมียที่มีความรุนแรงมากจำนวน 72 คน ด้วยเทคนิคการสร้างภาพแบบ bright และ black-blood ภาพได้รับการวิเคราะห์ที่สถาบันอ้างอิง และท้องถิ่นด้วยวิธีแบบจุดภาพ โดยคำนวณจากบริเวณกล้ามเนื้อหัวใจด้านผนังกั้นทั้งหมด (ที่สถาบันอ้างอิง) และบางส่วน (ที่สถาบันท้องถิ่น) ได้ทำการวิเคราะห์โดยใช้แบบจำลองแบบ offset ที่ทั้งสองสถาบันขณะที่ได้ใช้แบบ typical เพิ่มเติมที่สถาบันท้องถิ่น ความเปลี่ยนแปลงระหว่างสถาบันของค่า T2* ได้รับการวิเคราะห์โดยผู้สังเกตการณ์อิสระที่ไม่รู้ผลการทดลอง*

ผลการศึกษา: *ค่า T2* ที่ได้จากการวิเคราะห์ทั้งหมดมีค่าเปรียบเทียบกันได้ทางสถิติ ความเปลี่ยนแปลงระหว่างสถาบันเมื่อใช้แบบจำลองแบบ offset มีค่าร้อยละ 5.2 และ 4.4 สำหรับเทคนิคแบบ bright และ black-blood ตามลำดับ ซึ่งสูงกว่าความเปลี่ยนแปลงภายในสถาบันอยู่ประมาณร้อยละ 1.7 ค่าดังกล่าวอยู่ในย่านที่เทียบได้กับวิธีที่ได้รับการยอมรับซึ่งมีค่าประมาณร้อยละ 5.4 การเปรียบเทียบระหว่างแบบจำลองมีความเอนเอียงประมาณ 0.4 ms และมีการเปลี่ยนแปลงประมาณร้อยละ 6.9 และ 4.7 ตามลำดับ ค่าดังกล่าวมากกว่าค่าภายในสถาบันอยู่ร้อยละ 1.4*

สรุป: *ความเปลี่ยนแปลงจากผู้สังเกตการณ์ด้วยวิธีแบบจุดภาพโดยใช้แบบจำลองแบบ offset และ typical มีค่าเทียบเคียงกันได้ และใกล้เคียงกับค่าที่เคยมีการรายงานมาแล้ว ความเปลี่ยนแปลงระหว่างสถาบันก็อยู่ในระดับที่สมเหตุสมผล โดยมีค่ามากกว่าค่าภายในสถาบันอยู่น้อยกว่าร้อยละ 2 และมีความเอนเอียงประมาณ 0.4 ms*