Original Articles

Interlaboratory comparison for urinary trans, trans-muconic acid testing in Rayong province

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Abstract

Introduction: The objective of this study was to assess reliability of urinary trans, trans-muconic acid (t,t-MA) analysis by high performance liquid chromatography (HPLC) technique among three laboratories in Rayong province.

Method: We conducted the interlaboratory comparison among three laboratories for t,t-MA testing via two steps. Step one was the preparation phase to settle the agreement and protocol. Step two was the comparison: step 2.1 the comparative HPLC-analysis of blank and spiked urines and step 2.2 the comparative HPLC-analysis of 30 urine samples obtained from subjects. Subjects were gas station workers in Rayong province.

Result: The result obtained from step 2.1 showed the good percentage of recovery and comparable results. Non significant differences between each pair of the 3 laboratories and high intra-class correlation coefficients in step 2.2 implied good reliability of 3 laboratories.

Discussion and Conclusion: This interlaboratory comparison could be considered an initiative for external quality assurance of this laboratory testing.

Key words: Interlaboratory comparison, Intra-class correlation coefficients, Urine, Trans, trans-muconic acid, Benzene exposure biomarker, Rayong province

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Introduction

Benzene is a ubiquitous carcinogen[1] and air pollutant. One of the biomarkers of benzene exposure is urinary trans, trans-muconic acid (t,t-MA) which is an open-ringed or aliphatic metabolite[2] of benzene. t,t-MA was proposed to be a biomarker of environmental benzene exposure[3], but later found that it is also a metabolite of sorbic acid (2,4-hexadienoic acid)[4], a food preservative[5]. Nevertheless, t,t-MA testing is still performed in Thailand as a marker of benzene exposure, occupationally and environmentally, with no laboratory accreditation[6] for it. Different results from different laboratories may raise concerns about reliability and which one should be used. This prompts interlaboratory comparison of this testing.

Method

Accreditation process by conducting and interlaboratory comparison among three laboratories in Rayong Province (herein named Lab A, B, C) for urinary t,t-MA testing was initiated. This is a friendly and mutual process aiming to assess reliability and improve laboratory performance. This study was approved by the ethical committee of Rayong Hospital (Ryh-EC 2/2556). The interlaboratory comparison among three laboratories for urinary t,t-MA testing via two steps was conducted.

Step one: the preparation. Lab A initiated a relevant checklist and solicited opinions from other laboratories and three experts, made modifications and sent to all three laboratories and asked them to self-assess and send back to Lab A. After the experts' approval, Step two was proceeded.

Step two: the comparison. In step 2.1, all three laboratories analyzed urinary t,t-MA in blank and spiked urines. Lab A prepared one blank and three spiked urines (at concentrations of 0.1-7.0 mg/L), divided into three aliquots and sent to all three laboratories to analyze and find out percent recovery. Lab B and C officers came to pick up specimens from Lab A according to protocol. The specimens were kept at 2 - 8 ^{o}C during transportation and pre-analytical period, and all three laboratories reported the results to principal investigator within one week. The results and plotted graphs on pair-wise bases were analyzed.

After all laboratory results accorded with others, step 2.2 was proceeded. All three laboratories analyzed urinary t,t-MA in 30 subjects' specimens. The number of 30 based on rule of thumb for pilot study as discussed by Browne[7]. Lab A collected urine from 30 gas station workers in Amphur Meung, Rayong Province. Each urine specimen was divided into three aliquots, one for each lab to test for t,t-MA and creatinine. Again, Graphs on pair-wise bases were plotted and analyzed by paired t-test[8] and tested for intra-class correlation coefficients[9].

Result

All three laboratories analyzed t,t-MA with the method described by Boogaard and van Sittert[10] and analyzed creatinine by modified Jaffe method[11]. In step one, all laboratories accorded with others in qualification, method and practice of this test.

In step 2.1, tables 1-3 showed results of t,t-MA testing of blank and spiked urines of each laboratory. All three laboratories had percent recovery of higher than 88 which were considered good. R-square of each pair was higher than 0.99 indicating high correlation. These indicated comparable results of all three laboratories.

Table 1 Result of t,t-MA testing of blank and spiked urines of Lab A

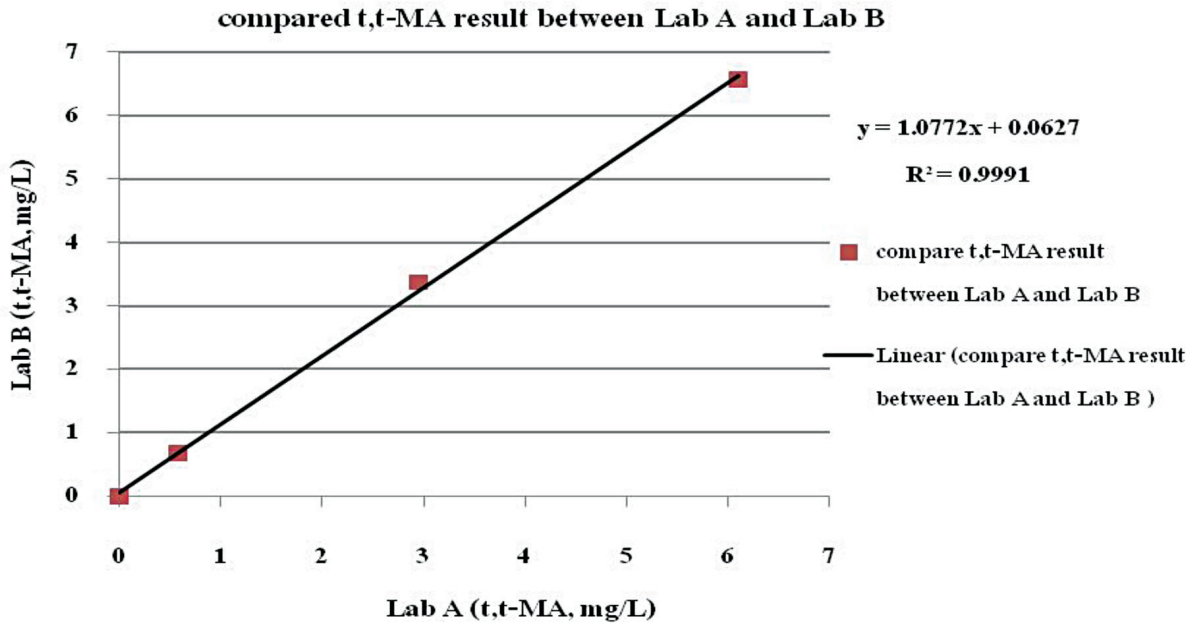
Sample	Predetermined t,t-MA concentration (mg/L)	Creatinine (mg/dl)	Test result of t,t-MA (mg/L)	Test result of t,t-MA (µg/gCr)	% Recovery
Blank urine	0.00	80.10	0.00	0.00	-
Spike urine 1	0.6272	76.50	0.5777	755.16	92.11
Spike urine 2	3.1360	74.50	2.9492	3958.66	94.04
Spike urine 3	6.2720	70.20	6.0987	8687.61	97.24

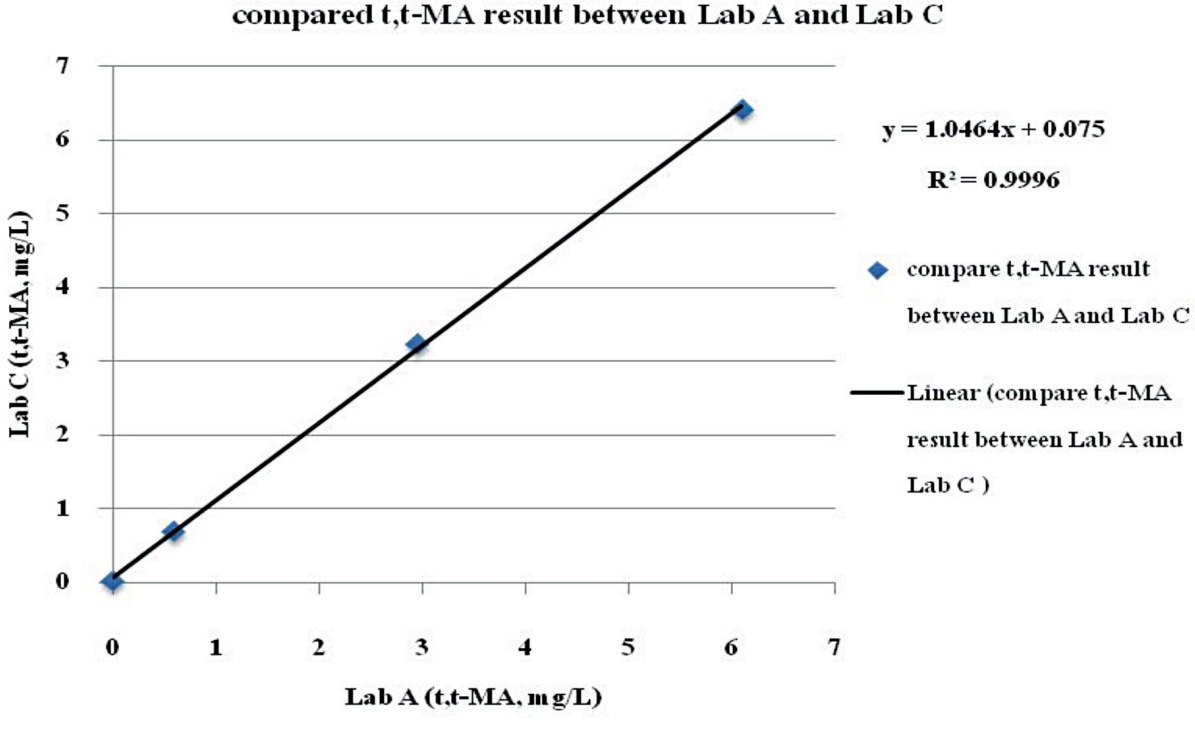
Table 2 Result of t,t-MA testing of blank and spiked urines of Lab B

Sample	Predetermined t,t-MA concentration (mg/L)	Creatinine (mg/dl)	Test result of t,t-MA (mg/L)	Test result of t,t-MA (µg/gCr)	% Recovery
Blank urine	0.00	79.02	0.00	0.00	-
Spike urine 1	0.6272	78.55	0.68	865.69	91.58
Spike urine 2	3.1360	77.78	3.37	4332.73	92.54
Spike urine 3	6.2720	74.26	6.57	8847.29	95.25

Table 3 Result of t,t-MA testing of blank and spiked urines of Lab C

Sample	Predetermined t,t-MA concentration (mg/L)	Creatinine (mg/dl)	Test result of t,t-MA (mg/L)	Test result of t,t-MA (µg/gCr)	% Recovery
Blank urine	0.00	84.57	0.01700	20.10	-
Spike urine 1	0.6272	81.16	0.69938	861.73	88.49
Spike urine 2	3.1360	77.83	3.23827	4160.70	96.74
Spike urine 3	6.2720	74.43	6.41734	8621.98	97.68


compared t,t-MA result between Lab A and Lab B
Lab B (t,t-MA, mg/L)
Lab A (t,t-MA, mg/L)
y = 1.0772x + 0.0627
R² = 0.9991
compare t,t-MA result between Lab A and Lab B
Linear (compare t,t-MA result between Lab A and Lab B)


compared t,t-MA result between Lab A and Lab C
Lab C (t,t-MA, mg/L)
Lab A (t,t-MA, mg/L)
y = 1.0464x + 0.075
R² = 0.9996
compare t,t-MA result between Lab A and Lab C
Linear (compare t,t-MA result between Lab A and Lab C)

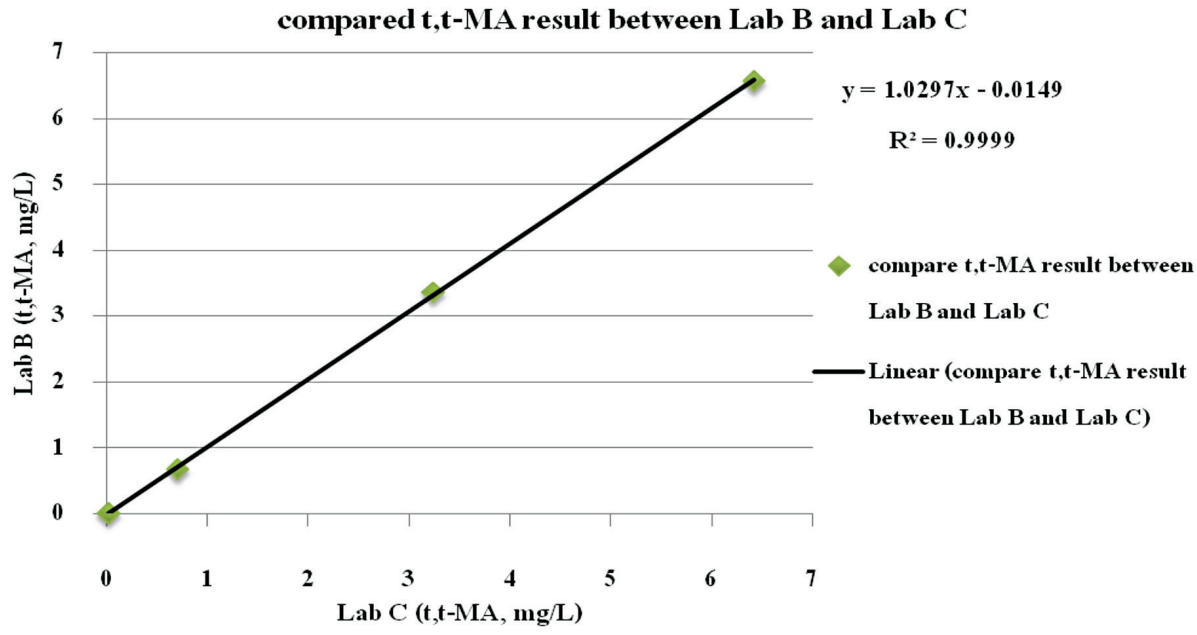


Figure 1 Line graphs showed correlations of three pairs of laboratories (blank and 3 spiked urines)

Table 4 showed characteristics of 30 subjects whose urines were used in step 2.2. Most (63.3%) of them were female, with mean age of 35.5 years, 53.3% finished high school, and 76.7% worked in fuel/gas filling task. Twenty six point seven percent of them had a pre-existing disease, most of them were asthma. Almost one-fourth (23.3%) smoked, and more than one-third (36.7%) drank alcohol. Regarding potential exposure to sorbic acid, all 30 subjects ate one or more of such food within two days prior to urine collection with fresh noodle as the most frequent one. As expected, 90% of them were occupationally exposed to organic solvents within two days prior to urine collection.

Table 4 Characteristics of subjects (30 gas station workers)

Characteristics	Number	Percent
Gender		
Male	11	36.7
Female	19	63.3
Age		
Less than 20	4	13.3
21 – 30	8	26.7
31 – 40	6	20.0
41 – 50	8	26.7
51 – 60	3	10.0
Older than 60	1	3.3

Table 4 Characteristics of subjects (30 gas station workers) (continue)

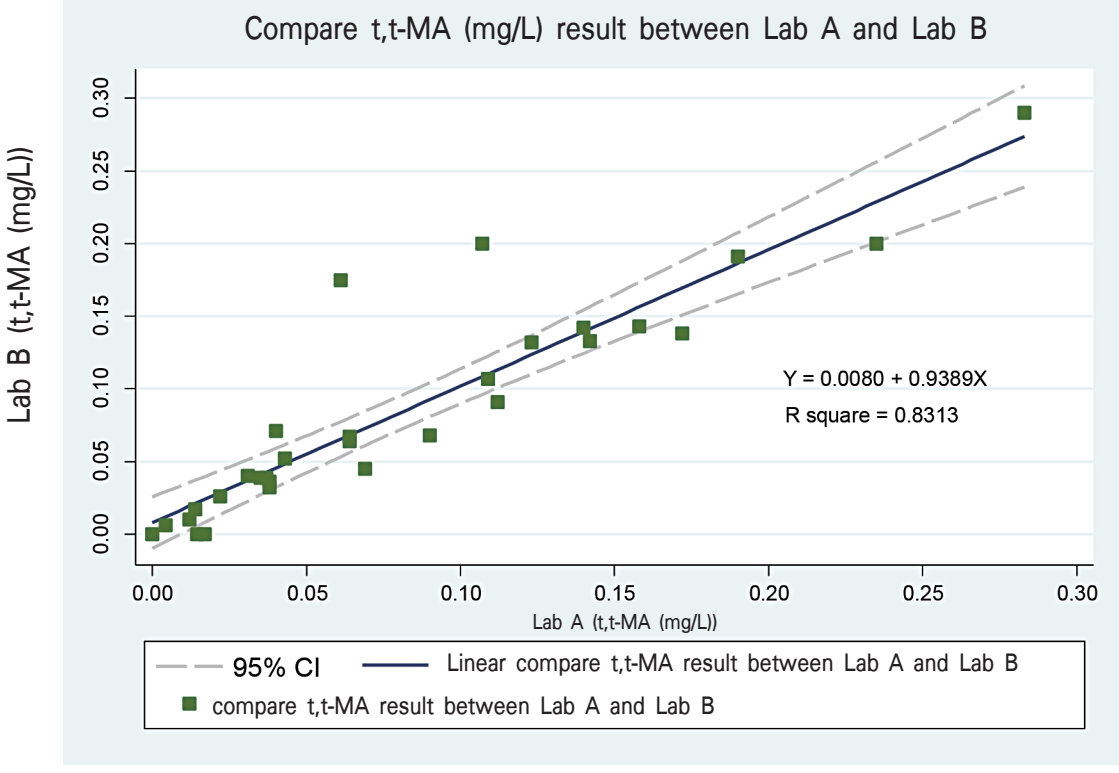
Characteristics	Number	Percent
Mean 35.5, Min 17, Max 65 years old		
Educational level		
Primary	6	20.0
Secondary	16	53.3
Diploma	5	16.7
Bachelor	2	6.7
Higher than bachelor	1	3.3
Work section		
Garden	1	3.3
Fuel/gas filling	23	76.7
Office	6	20.0
Pre-existing disease diagnosed by physician		
No	22	73.3
Yes (may reply more than 1)	8	26.7
Asthma	5	16.6
Diabetes	1	3.3
Hypertension	1	3.3
Thyroid	1	3.3
Cataract	1	3.3
Smoking		
No	23	76.7
Yes	7	23.3
Alcohol drinking		
No	19	63.3
Yes	11	36.7
Consumption of listed food within 2 days prior to urine collection		
No	0	0.0
Yes (may reply more than 1)	30	100.0
Fresh noodle	16	53.3
Sausage	14	46.7
Shrimp paste	13	43.3
Instant noodle	13	43.3
Cheese/margarine/mayonnaise	8	26.7
Preserved food/fruit	7	23.3
Canned/boxed juice	6	20.0
Salted food/vegetable	6	20.0
Dried fish	6	20.0
Canned food	5	16.7
Carbonated drink	1	3.3

Table 4 Characteristics of subjects (30 gas station workers) (continue)

Characteristics	Number	Percent
Work exposure to organic solvent within 2 days prior to urine collection		
No	3	10.0
Yes (may reply more than 1)	27	90.0
Diesel/gasoline	24	80.0
Air conditioner spray	10	33.3
Insecticide	4	13.3
Paint/thinner	1	3.3

Results of step 2.2 were showed in figures 2-3, and tables 5-6. Figure 2 showed correlations of three pairs of laboratories in terms of t,t-MA, while figure 3 showed those of t,t-MA/creatinine. Table 5 showed comparisons of t,t-MA (mg/L) of each pair and inferential statistics (paired t-test), while table 6 showed those of t,t-MA adjusted by creatinine (μg/gCr). Each pair had good correlation and no significant differences. Table 7 showed high intra-class correlation coefficients among the three laboratories, 0.849 for t,t-MA and 0.713 for t,t-MA/Cr, respectively. These indicated that all three laboratories had good rater reliability on t,t-MA testing.

As expected, subjects who did the fuel/gas filling work had higher t,t-MA than those who did garden or office work (mean ± SD 0.038 ± 0.0137 vs 0.096 ± 0.0771 mg/L by Lab A, p-value 0.003 by independent t-test). But there were not any correlations between urinary t,t-MA and number of potential sorbic acid-contained food consumption (Pearson's correlation coefficient −0.026, p-value 0.89).



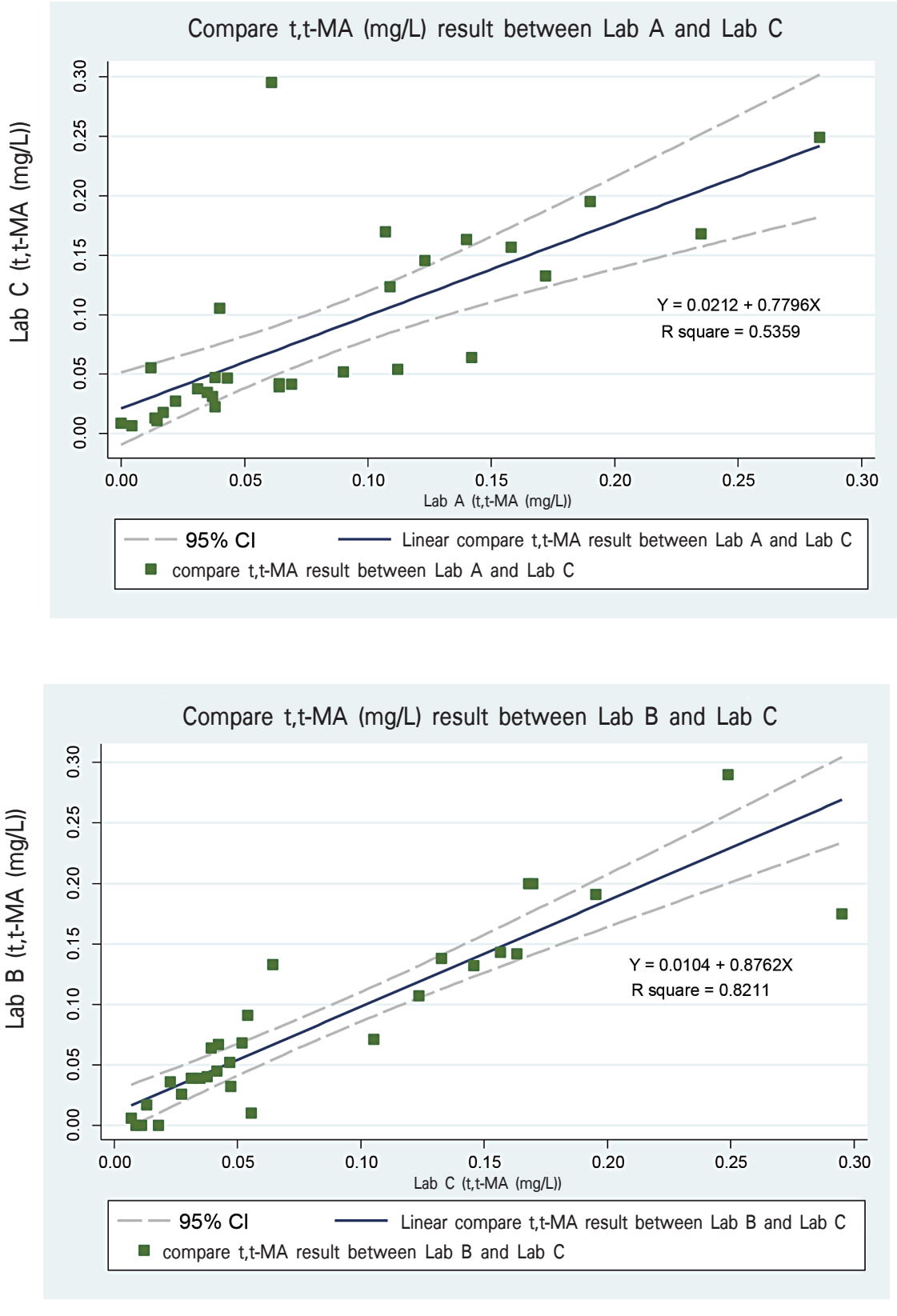
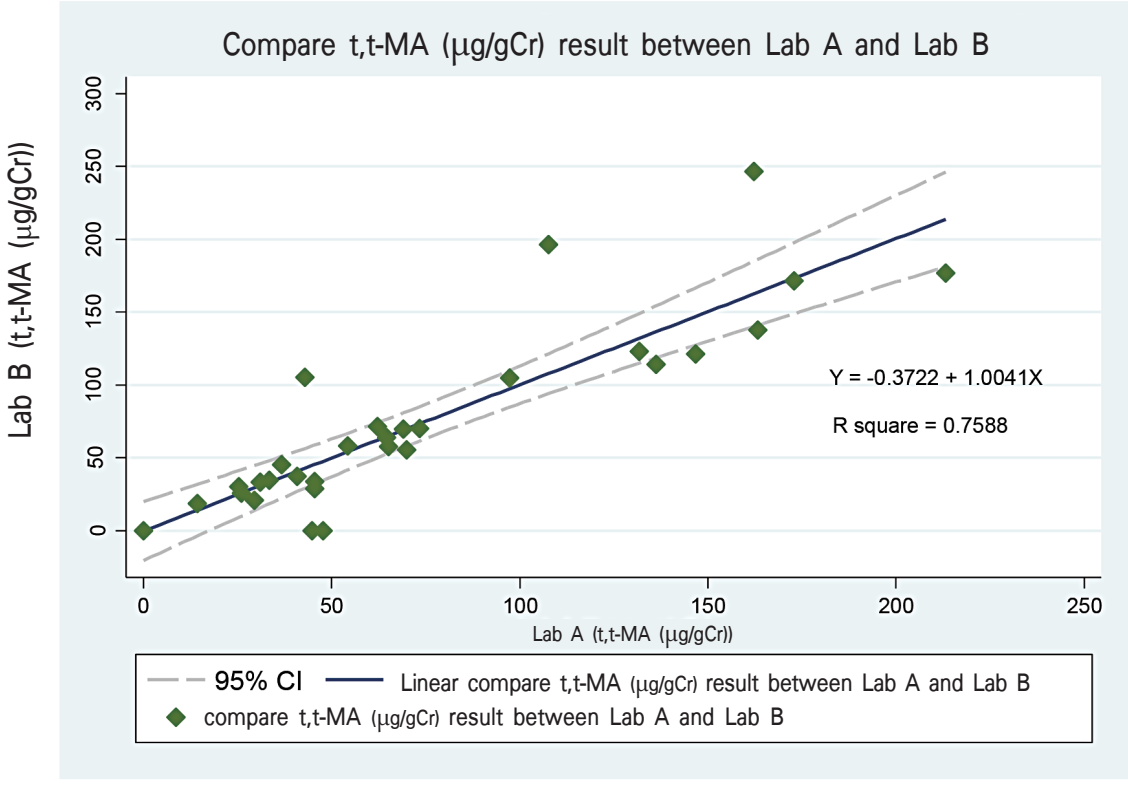
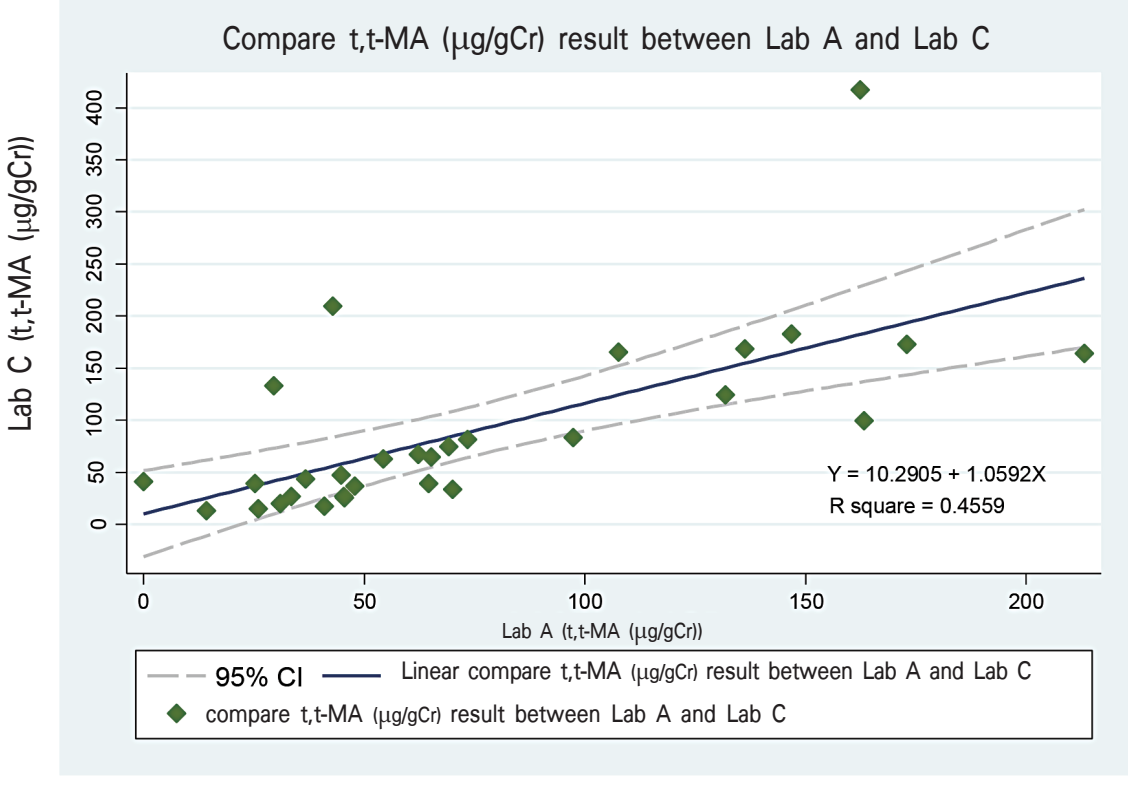


Figure 2 Line graphs showed correlations of three pairs of laboratories (t,t-MA of 30 subjects' urine)


Compare t,t-MA (µg/gCr) result between Lab A and Lab B
Lab B (t,t-MA (µg/gCr))
0
50
100
150
200
250
300
Y = -0.3722 + 1.0041X
R square = 0.7588
0
50
100
150
200
250
Lab A (t,t-MA (µg/gCr))
95% CI
Linear compare t,t-MA (µg/gCr) result between Lab A and Lab B
compare t,t-MA (µg/gCr) result between Lab A and Lab B


Compare t,t-MA (µg/gCr) result between Lab A and Lab C
Lab C (t,t-MA (µg/gCr))
0
50
100
150
200
250
300
350
400
Y = 10.2905 + 1.0592X
R square = 0.4559
0
50
100
150
200
Lab A (t,t-MA (µg/gCr))
95% CI
Linear compare t,t-MA (µg/gCr) result between Lab A and Lab C
compare t,t-MA (µg/gCr) result between Lab A and Lab C

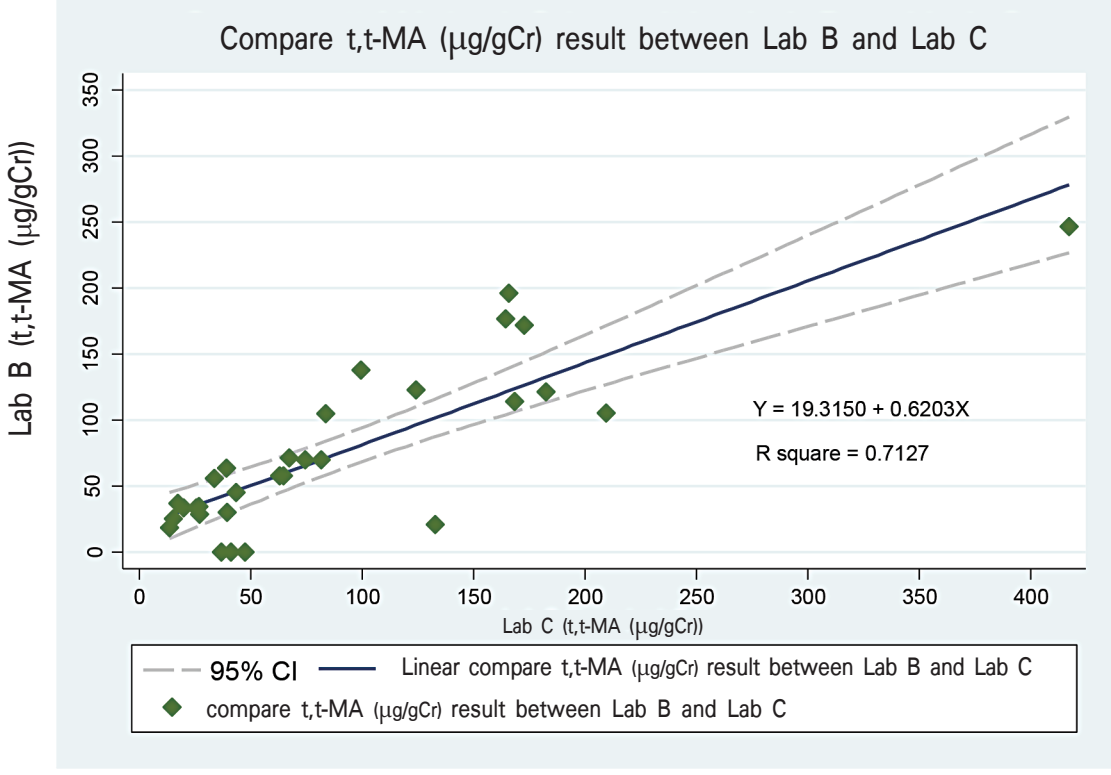


Figure 3 Line graphs showed correlations of three pairs of laboratories (t,t-MA/creatinine of 30 subjects' urine)

Table 5 Comparison of t,t-MA (mg/L) of each laboratory pair and inferential statistics (paired t-test) (n=30)

Laboratory	Mean	SD	T	p-value
Lab A	0.082	0.072	-0.529	0.600
Lab B	0.090	0.074		
Lab A	0.082	0.072	-0.309	0.759
Lab C	0.085	0.077		
Lab B	0.090	0.074	0.018	0.985
Lab C	0.085	0.077		

Table 6 Comparison of t,t-MA adjusted by creatinine (µg/gCr) of each laboratory pair and inferential statistics (paired t-test) (n=30)

Laboratory	Mean	SD	t	p-value
Lab A	75.13	54.351	0.012	0.990
Lab B	75.06	62.650		
Lab A	75.13	54.351	-1.282	0.210
Lab C	89.87	85.263		
Lab B	75.06	62.650	-1.739	0.093
Lab C	89.87	85.263		

Table 7 Intra-class correlation coefficients (ICC) of t,t-MA (mg/L) and t,t-MA adjusted by creatinine (μg/gCr) among three laboratories

	N	Lab A mean ± SD	Lab B mean ± SD	Lab C mean ± SD	ICC	95%CI	p-value
t,t-MA	30	0.082 ± 0.072	0.090 ± 0.074	0.085 ± 0.077	0.849	0.745, 0.919	< 0.001
t,t-MA/Cr	30	75.13 ± 54.351	75.06 ± 62.650	89.87 ± 85.263	0.713	0.546, 0.838	< 0.001

Discussion and Conclusion

The interlaboratory comparison among three laboratories in Rayong Province for urinary t,t-MA testing was conducted. The results revealed that all three laboratories' performance on t,t-MA testing was in good agreement and reliable.

This study had some strength. First, it was a good initiation of co-operation between governmental and private agencies, although they were competing with the others in providing laboratory services. Their customers or patients will eventually benefit from their laboratory performance improvement. Second, the chemists/analysts of each laboratory were blinded on information of urine's subjects. This helped reducing bias.

This study also had several weaknesses. First, there was no measurement of sorbic acid in any food surveyed nor ambient air benzene in the gas station. Second, Subjects were not asked to refrain from eating potential sorbic acid-contained food a couple days before urine collection since this study was not designed for biological monitoring. Third, the number of potential sorbic acid-contained food consumption within two days prior to urine collection was used as simple surrogate of sorbic acid exposure. This was likely to cause non-differential misclassification bias and may partly explain why there were not any correlations between urinary t,t-MA and number of potential sorbic acid-contained food consumption.

Although all three laboratories' performance on t,t-MA testing was in good agreement, the graphs obviously showed that there were some discrepancies. This was similar to an interlaboratory exercise[12] showing scattering of quantitative data even among accredited laboratories. However, we consider these as rooms for improvements for our laboratories.

This interlaboratory comparison study could be considered an initiation for external quality assurance process leading to laboratory accreditation on t,t-MA testing in the future. Although this study could not claim for validity, it showed good rater reliability that should be secured before assessing validity. Continuous improvement for t,t-MA testing performance of each laboratory was discussed and agreed. In the future, specialized analyses like the Me. Tos. Project in Italy might be considered[13].

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บทคัดย่อ

เปรียบเทียบการตรวจ trans, trans-muconic acid ในปัสสาวะ ระหว่างห้องปฏิบัติการในจังหวัดระยอง

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บทนำ: การศึกษานี้มีวัตถุประสงค์เพื่อประเมินความแม่นยำของการตรวจกรดทรานส์ ทรานส์ – มิวโคนิค (t,t-MA) ในปัสสาวะด้วยเทคนิค high performance liquid chromatography (HPLC) ระหว่างห้องปฏิบัติการสามแห่งในจังหวัดระยอง

วิธีการศึกษา: ผู้วิจัยทำการศึกษาเปรียบเทียบการตรวจดังกล่าวเป็นสองระยะ ระยะแรกเป็นขั้นเตรียมการโดยกำหนดข้อตกลงและขั้นตอนการศึกษาร่วมกัน ระยะที่สองเป็นขั้นเปรียบเทียบ ระยะ ๒.๑ เป็นการใช้เทคนิค HPLC วิเคราะห์ตัวอย่างปัสสาวะที่ไม่มีสาร (blank) และที่ใส่สาร t,t-MA ที่ทราบความเข้มข้น (spiked) ระยะ ๒.๒ เป็นการใช้เทคนิค HPLC วิเคราะห์ปัสสาวะ ๓๐ ตัวอย่างจากพนักงานสถานีบริการน้ำมันในจังหวัดระยอง

ผลการศึกษา: ผลของระยะ ๒.๑ แสดงให้เห็นว่าห้องปฏิบัติการทั้งสามสามารถตรวจกรดทรานส์ ทรานส์ – มิวโคนิคที่ใส่ลงไปได้ (recovery) ในสัดส่วนที่สูงและผลคล้ายคลึงกัน ระยะ ๒.๒ ไม่พบความแตกต่างอย่างมีนัยสำคัญเมื่อพิจารณาผลของห้องปฏิบัติการทีละคู่ และค่าสัมประสิทธิ์สหสัมพันธ์ระหว่างชั้น (intra-class correlation coefficients) ที่สูง บ่งชี้ว่าห้องปฏิบัติการทั้งสามมีความแม่นยำในการตรวจ t,t-MA

วิจารณ์ และ
สรุปผลการศึกษา: การศึกษาเปรียบเทียบนี้อาจเป็นการเริ่มต้นการประกันคุณภาพห้องปฏิบัติการในการตรวจสารนี้

คำสำคัญ: การเปรียบเทียบระหว่างห้องปฏิบัติการ, ค่าสัมประสิทธิ์สหสัมพันธ์ระหว่างชั้น, ปัสสาวะ, กรดทรานส์ ทรานส์ – มิวโคนิค, ตัวบ่งชี้การสัมผัสเบนซีน, จังหวัดระยอง