RESEARCH ARTICLE

Performance evaluation of five commercial assays for detection of acetaminophen

Bao-Yum Chan¹ | Hing-Man Tsang¹ | Candy Wai-Yan Ng² | William Hin-Wing Ling¹ | Daniel Cheuk-Wa Leung² | Hencher Han-Chih Lee² | Chloe Miu Mak²

¹Department of Pathology, Clinical Laboratory, North Lantau Hospital, Lantau, Hong Kong

²Department of Pathology, Chemical Pathology Laboratory, Princess Margaret Hospital, Kowloon, Hong Kong

Correspondence

Chloe Miu Mak, Department of Pathology, Chemical Pathology Laboratory, Princess Margaret Hospital, Kowloon, Hong Kong.
Email: makm@ha.org.hk

Background: To evaluate the analytical performance of five commercial acetaminophen assays and select the best method for routine use.

Methods: Imprecision, accuracy, linearity, and interferences of three enzymatic assays (Beckman Coulter AU Paracetamol, Abbott MULTIGENT Acetaminophen, and Sekisui Acetaminophen L3K) and two immunoassay-based assays (Beckman Coulter SYNCHRON ACTM (Acetaminophen) Reagent and Siemens SYVA Emit-tox Acetaminophen) were evaluated on a Beckman Coulter AU680 chemistry analyzer. Hook effect for immunoassay-based assays and recovery in ultrafiltrate for enzymatic methods were studied.

Results: Within-run and between-run imprecision of the enzymatic assays ranged 0.26%-0.82% and 0.53%-2.86%, respectively, while that for the immunoassay-based methods ranged 0.96%-6.34% and 1.50%-11.33%, respectively. All assays except the SYNCHRON assay fell within the program analytical performance specifications ($\pm 20 \mu\text{mol/L}$ or 10%) for external quality assurance (EQA) samples, with the highest positive bias (31.7%) observed in the SYNCHRON assay. Icteric interference was demonstrated most significantly in the Abbott assay (up to $88 \mu\text{mol/L}$ positive bias in blank serum). The lipemic interference on the SYNCHRON was significant (up to 110% positive bias at level of $100 \mu\text{mol/L}$). The immunoassay-based methods were less susceptible to hemolytic interference, while the Abbott and AU assays were more susceptible to N-acetylcysteine interference. Both immunoassay-based methods showed no hook effect up to $18\,000 \mu\text{mol/L}$. Ultrafiltration recoveries for enzymatic methods were satisfactory, ranging from $80.0\% \pm 5.1\%$ to $89.5\% \pm 3.0\%$.

Conclusions: Proportional bias was observed in the SYNCHRON assay, while the Siemens and Sekisui assays were minimally affected by bilirubin interferences.

KEYWORDS

Acetaminophen, enzymatic assay, evaluation, immunoassay

1 | INTRODUCTION

Acetaminophen (N-acetyl-para-aminophenol, paracetamol) is one of the most frequently used analgesic and antipyretic medications worldwide and is believed to act mainly through inhibition of cyclooxygenase enzyme and reduce prostaglandin synthesis.¹ It is a non-prescription medication and is available as a single entity product, or as a compound formula, for example, with other flu medications. Because of its popularity and accessibility, acute intentional and chronic unintentional acetaminophen overdoses are relatively common. However, given the lack of symptoms, or relative non-specificity of symptoms in the initial phase, acetaminophen poisoning is difficult to diagnose solely based on clinical findings. Without prompt diagnosis and treatment, those patients would, later on, develop severe hepatotoxicity and mortality may result.²

Serum acetaminophen level, interpreted with the modified Rumack-Matthew nomogram in relation to the time after ingestion, is the most important biochemical test for diagnosing acute poisoning and for stratifying those who are at higher risk of developing clinical toxicity and thus justifying the use of antidote. The potentially fatal hepatic injury caused by acetaminophen toxicity would be largely prevented by timely administration of antidote, N-acetyl cysteine (NAC), guided by serum acetaminophen levels.³ Serious hepatotoxicity or mortality is uncommon if the antidote can be administered within eight hours post-overdose.

Because of the relatively high prevalence of acetaminophen poisoning in general population, non-specificity of early presenting signs and symptoms, and the significant yet preventable toxicity of acetaminophen, routine checking of serum acetaminophen in every patient presenting with a known intentional self-harm, suicidal attempts, or suspected intentional or unintentional overdose are recommended. Therefore, an efficient and reliable round-the-clock emergency service for serum acetaminophen quantitative testing should be available in every major hospital in view of the urgency in identifying and treating such patients.

The automated acetaminophen assays used by clinical laboratories can be broadly divided into two groups: the commonly used enzymatic assays and newer immunoassay-based assays.

The enzymatic assays have advantages of convenient and rapid compared to chromatographic methods, and being more economical compared to immunoassay-based assays. However, their performance is limited by different interfering substances such as bilirubin and hemoglobin in patient samples.⁴ The immunoassay-based assays are generally less affected by bilirubin, hemoglobin, and also NAC interferences.⁵

In this study, we evaluate the analytical performances of three enzymatic acetaminophen assays, including Beckman Coulter AU Paracetamol, Abbott MULTIGENT Acetaminophen, and Sekisui Acetaminophen L3K, and two immunoassay-based acetaminophen assays, including Beckman Coulter SYNCHRON ACTM (Acetaminophen) Reagent and Siemens SYVA Emit-tox

Acetaminophen, which is available in the market and adoptable for Beckman Coulter AU680 automated chemistry analyzer.

Beckman Coulter SYNCHRON ACTM (Acetaminophen) Reagent (immunoassay-based method) was originally selected for Beckman Coulter AU680 chemistry analyzer in the laboratory. Since the performance of existing method in external quality assurance (EQA) program was unsatisfactory with high bias, alternate method with better analytical performance was sought for the analyzer. Various market available reagent kits of both enzymatic and immunoassay-based assays were explored, and five adoptable assay kits were selected for this study.

With the opportunity and resources to study five commercial assay kits simultaneously from independent researchers, this study provides objective and comprehensive information for the laboratory to select the most suitable method for acetaminophen determination on existing chemistry autoanalyzer.

2 | MATERIAL AND METHODS

The evaluation study was carried out in the clinical laboratory of a satellite hospital with 24-hour emergency department service in Hong Kong. Five commercial acetaminophen assay kits were evaluated on a Beckman Coulter AU680 chemistry analyzer (Beckman Coulter KK, Tokyo, Japan) from March to June 2015.

Analytical performances of the five acetaminophen assay were evaluated in terms of imprecision, accuracy, and linearity. The effect of endogenous interferences, such as hemolysis, lipemia, and icterus samples, and the exogenous interferences, such as NAC (as antidote for acetaminophen toxicity), was studied. Left-over patient serum samples without obvious hemolysis, icterus, or lipemia (with normal serum indices) from patients who did not have history of recent acetaminophen intake were used as control blank serum. Furthermore, recovery of analyte in ultrafiltrate, after ultrafiltration for removing small interfering substances including protein-bound bilirubin, hemoglobin, and lipoprotein, was evaluated for the enzymatic methods, while further experiments were performed on the immunoassay-based methods to look for possible hook effects.

Three enzymatic methods were included in the study: Beckman Coulter AU Paracetamol (Beckman Coulter Inc, Brea, CA, USA, lot number 5817), Abbott MULTIGENT Acetaminophen (Abbott Laboratories, Abbott Park, IL, USA, lot number 46953UQ12), and Sekisui Acetaminophen L3K (Sekisui Diagnostics, Charlottetown, Canada, lot number 47695), in which acetaminophen was broken down by aryl-acylamidase to p-aminophenol, which was further coupled with different phenolic compounds (o-cresol, p-xenol, and 5-hydroxyquinoline sulfate, respectively) in the presence of oxidizing agents. Moreover, two immunoassay-based methods, namely Beckman Coulter SYNCHRON Systems ACTM (Acetaminophen) Reagent (Beckman Coulter Inc, Brea, CA, USA, lot number M401188) and SYVA Emit-tox Acetaminophen (Siemens Healthcare Diagnostics

Inc, Newark, DE, USA, lot number 7A19UL-G3), which utilize particle-enhanced turbidimetric inhibition immunoassay (PETINA) and enzyme multiplied immunoassay technique (EMIT), respectively, were also evaluated.

2.1 | Imprecision & accuracy

Three acetaminophen control materials (Liquid Unassayed Multiqual, Bio-Rad Laboratories, Irvine, CA, USA) at low, medium, and high levels of 99, 331, and 992 $\mu\text{mol/L}$, respectively, were used. All of them were analyzed in triplicate on each day for five days. The within-run and between-run coefficients of variation (CV) were calculated according to Clinical and Laboratory Standards Institute (CLSI) Guideline EP05, Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline.

Twelve samples from condensed general chemistry program cycle 98 and 99 of quality assurance program (QAP) materials from the Royal College of Pathologists of Australasia (RCPA) were analyzed on all of the five acetaminophen assays, and their results were plotted against the overall median values and compared with the program analytical performance specifications (APS; ± 20 up to 200 $\mu\text{mol/L}$; 10% if >200 $\mu\text{mol/L}$) to determine the accuracy of each assay.

2.2 | Linearity

A control blank serum sample was spiked with standard a solution of acetaminophen with serial dilution, to yield final concentrations of 400, 800, 1200, 1600, 2000, 2400, 2800, and 3200 $\mu\text{mol/L}$. They were then measured with the five acetaminophen assays. Each of the measured values by each assay was plotted against the calculated concentration to determine the linearity range of each assay, and the regression equations were calculated to determine the coefficient of correlation, slope, and intercept values. Variations from calculated value within the RCPA APS (± 20 up to 200 $\mu\text{mol/L}$ or 10% if >200 $\mu\text{mol/L}$) were regarded as acceptable value.

A control blank serum sample was spiked with paracetamol to 20 $\mu\text{mol/L}$ and analyzed 10 times for the determination of limit of quantitation. Manufacturers' claim from the five assays was compared and verified. Percentage of recovery of serially diluted samples was calculated.

2.3 | High-dose hook effect

Control blank serums were further spiked with acetaminophen to yield final acetaminophen concentration of 18 000 $\mu\text{mol/L}$, which was then measured with the two immunoassay-based assays to look for the presence of possible high-dose hook effect.

2.4 | Interference studies

Common interferences such as lipemia, hemolysis, bilirubinemia, and NAC were included in this part of study. Variations from non-spiked

sample by interference spiked sample within the RCPA APS (± 20 up to 200 $\mu\text{mol/L}$ or 10% if >200 $\mu\text{mol/L}$) were regarded as acceptable value without significantly affected by specific interfering substance.

2.4.1 | Lipemia

Control blank serums were spiked with acetaminophen and different volumes of 20% Intralipid (Fresenius Kabi, Sweden), yielding final acetaminophen concentration of 100 $\mu\text{mol/L}$ and Intralipid concentrations of 0, 2.26, 4.52, 6.78, and 11.3 mmol/L, which were then measured with the five acetaminophen assays.

The concentrations of Intralipid were calculated by dilution of the original solution. The solution provides opacity similar to triglyceride or lipid which interferes with the assays.

2.4.2 | Hemolysis

Control blank serums were spiked with acetaminophen and different volumes of own preparation of red blood cell lysate, yielding final acetaminophen concentration of 100 $\mu\text{mol/L}$ and hemoglobin concentrations of 0, 2, 4, 6, and 10 g/L, which were then measured with the five acetaminophen assays.

2.4.3 | Bilirubinemia

Control blank serum and sera of jaundiced patients with I-index of N (<43 $\mu\text{mol/L}$), 1+ (43–85 $\mu\text{mol/L}$), 2+ (86–172 $\mu\text{mol/L}$), 3+ (173–341 $\mu\text{mol/L}$), and 4+ (342–684 $\mu\text{mol/L}$) were included for this experiment. Each of these samples was spiked with acetaminophen to final concentrations of 0, 100, and 1000 $\mu\text{mol/L}$ and assayed.

Bilirubin interference is most concerned in enzymatic acetaminophen assays and was repeatedly reported in the literature.^{4,6,7} Two concerns of bilirubin interference, the yellow pigment affecting absorption at near the same wavelength (around 600 nm) and the reduction property, may have different effects at low and high acetaminophen concentrations. Therefore, checking bilirubinemia for different acetaminophen concentrations was performed in this study.

2.4.4 | NAC interference (at low and high acetaminophen concentrations)

Control blank serums were spiked with acetaminophen and Hidonac (5 g of NAC in 25 mL, Zambon, Vicenza, Italy), yielding final acetaminophen concentrations of 100 or 1000 $\mu\text{mol/L}$ and NAC concentrations of 0, 3.06, 6.13, 9.19, and 12.3 mmol/L, and were then measured with the five acetaminophen assays.

2.4.5 | Interference due to sample matrix

Copper sulfate was used for color development in Beckman Coulter AU Paracetamol assay. Proteins in the sample may participate in the reaction and hence cause the matrix interference. To evaluate the possible interference due to sample matrix in the AU assay, samples

TABLE 1 Summary of the results of the different acetaminophen assays

Assay	Within-run CV (%) ^a	Between-run CV (%) ^a	Accuracy ^b	Linear range ^c	Regression equation	R ²
AU	0.49, 0.50, 0.30 (0.7, 0.5, 0.4)	1.36, 0.98, 0.58 (3.5, 1.6, 0.9)	12/12 (−2.0 to 4.6)	47–3175 (99–2500)	$y = 0.9919x + 54.337$	0.9992
MULTIGENT	0.55, 0.26, 0.26 (0.6, 0.4, 0.3)	2.86, 0.94, 0.74 (1.9, 0.8, 1.1)	12/12 (−10.7 to −6.0)	19–3066 (20–2500)	$y = 0.9674x + 19.358$	0.9994
L3K	0.82, 0.51, 0.42 (1.5, 0.8, 0.6)	1.60, 1.28, 0.53 (2.9, 1.3, 1.3)	12/12 (−10.7 to 7.9)	19–3250 (4–2500)	$y = 1.0239x + 20.911$	0.9994
ACTM	6.34, 1.42, 0.96 (4.3, 2.4, 2.1)	11.33, 3.42, 1.50 (10.0, 4.6, 4.4)	0/12 (20.0 to 36.0)	75–1600 (66–1986)	$y = 1.2838x + 44.73$	0.9994
SYVA	2.37, 2.13, 2.88 (3.6, 2.5, 3.8)	5.53, 4.72, 5.96 (4.4, 4.1, 5.0)	12/12 (−16.2 to −0.1)	19–2425 (0.8–1324)	$y = 1.015x + 0.8571$	0.9999

ACTM, Beckman Coulter SYNCHRON Systems ACTM; AU, Beckman Coulter AU Paracetamol; CV, coefficient of variation; L3K, Sekisui Acetaminophen L3K; MULTIGENT, Abbott MULTIGENT Acetaminophen; R², coefficient of determination; SYVA, Siemens SYVA Emit-tox Acetaminophen

^aThree acetaminophen control materials at levels of 99, 331, and 992 $\mu\text{mol/L}$ were used. Values in bracket refer to CV claimed by the corresponding manufacturers.

^bResults of the twelve samples of RCPA condensed general chemistry program (sample with RCPA median value in $\mu\text{mol/L}$: 98-01:1117, 98-02:112, 98-05:436, 98-06:719, 98-09:866, 98-10:583, 98-13:111, 98-14:1116, 99-01:430, 99-02:715, 99-05:585, 99-06:873) within analytical performance specification (APS; ± 20 up to 200 $\mu\text{mol/L}$; 10% if >200 $\mu\text{mol/L}$) are shown. The QAP sample with range of bias in % is shown in brackets.

^cRanges from limit of quantitation to validated upper limit of linearity for each assay are shown. Values in bracket refer to linear range claimed by the corresponding manufacturers.

with various concentrations of total protein from patients without exposure to acetaminophen were measured by this assay.

2.4.6 | Recovery of acetaminophen after ultrafiltration

Elimination of bilirubin interference by ultrafiltration from sample prior to measuring acetaminophen with enzymatic assay was suggested in the literature.⁴ The three quality control materials of low, medium, and high acetaminophen levels (Liquid Unassayed Multiquel, Bio-Rad Laboratories) were measured before and after ultrafiltration with NanoSep 10 K (MW) OMEGA sample filters (Gelman, Port Washington, NY, USA) at 14 000 g for 15 minutes according to the recommendations by manufacturer. Acetaminophen levels in the pre- and post-treatment samples were measured with the three enzymatic acetaminophen assays, and percentage of recovery was calculated and compared accordingly.

3 | RESULTS

3.1 | Imprecision & accuracy

Results of within-run and between-run imprecision study of the five acetaminophen assays are summarized in Table 1. The precision profile of each assay derived from this study showed comparable results to the claims by the corresponding manufacturers. In general, enzymatic methods (Beckman Coulter AU, Abbott and Sekisui) showed better within-run and between-run precision than the immunoassay-based methods (Beckman Coulter SYNCHRON and Siemens).

The results of the accuracy study are summarized in Table 1 and Figure 1. All but one acetaminophen assay showed satisfactory

accuracy, and all the results of 12 RCPA samples fell within the RCPA APS, while for Beckman Coulter SYNCHRON (immunoassay-based assay) the results showed a significant high bias beyond the APS when compared to the RCPA median value.

FIGURE 1 Accuracy of the five acetaminophen assays on 12 samples of RCPA QAP condensed general chemistry program cycle 98 and 99. ACTM, Beckman Coulter SYNCHRON Systems ACTM; AU, Beckman Coulter AU Paracetamol; L3K, Sekisui Acetaminophen L3K; MULTIGENT, Abbott MULTIGENT Acetaminophen; SYVA, Siemens SYVA Emit-tox Acetaminophen

3.2 | Linearity

The results of the linearity study are summarized in Tables 1 and 2. All acetaminophen assays showed good linearity up to acetaminophen concentrations of around 2500–3000 $\mu\text{mol/L}$, consistent with the analytical ranges as claimed by the manufacturers, except for the Beckman Coulter SYNCHRON system ACTM, in which the linearity range as demonstrated by this study (1600 $\mu\text{mol/L}$) was lower than the claimed value of 1986 $\mu\text{mol/L}$.

For spiked sample with acetaminophen level of lower as 20 $\mu\text{mol/L}$, the Abbott, Sekisui, and Siemens assays showed acceptable performance within the APS and determined as limit of quantitation, while high bias was demonstrated by Beckman Coulter AU (mean 46.59 $\mu\text{mol/L}$) and SYNCHRON (mean 75.17 $\mu\text{mol/L}$) assays. Since the claimed lower limits of linearity of the AU and SYNCHRON assays were 99 and 66 $\mu\text{mol/L}$, respectively, the performance for such low concentration of acetaminophen is for reference only.

Like other therapeutic drugs, reference range is not applicable for acetaminophen. For clinical concern of acetaminophen overdose, modified Rumack-Matthew nomogram is often referred.³ The measured acetaminophen concentration and hours after ingestion should be correlated and considered for the treatment of patient with overdose of this drug.

3.3 | High-dose hook effect

The responses for serial dilutions measured by both immunoassay-based assays were linear, and no high-dose hook effect was demonstrated for both assays up to 18 000 $\mu\text{mol/L}$. This concentration is unlikely to be encountered in practical situations but can be served as information for the robustness of recent immunoassay-based assays eliminating such phenomenon.

3.4 | Interference study

3.4.1 | Lipemic interference

The results of lipemic interference study of the five acetaminophen assays are summarized in Table 3. All of the enzymatic assays (Beckman Coulter AU, Abbott, and Sekisui) and the Siemens assay

did not show significant lipemic interference up to 6.78 mmol/L of Intralipid spiked with less than 20 $\mu\text{mol/L}$ difference from non-lipemic sample. The Beckman Coulter SYNCHRON assay claimed not to be affected by lipemia of up to 4+ (approximately 4.07–4.52 mmol/L); however, our experiment demonstrated a dose-dependent positive interference by Intralipid, where the SYNCHRON assay was affected by Intralipid level as low as 2.26 mmol/L with more than 20 $\mu\text{mol/L}$ difference from non-lipemic sample.

3.4.2 | Hemolysis interference

The results of hemolytic interference study of the five acetaminophen assays are summarized in Table 3. The two immunoassay-based assays were virtually free from hemolytic interference despite the positive bias in Beckman Counter SYNCHRON assay. There was no significant interference at hemoglobin level up to 2.0 g/L for the three enzymatic methods at acetaminophen level of 100 $\mu\text{mol/L}$; however, beyond this level, a dose-dependent positive interference was observed with more than 20 $\mu\text{mol/L}$ difference from non-hemolysis sample, and all three assays were affected at a similar magnitude.

3.4.3 | Bilirubin interference

It is known that icteric samples would cause false-positive results in blank serum samples in some of the assays.⁶ The Abbott assay was the one most susceptible to icterus interference among the five, with the measured acetaminophen concentration increasing from 1.3 $\mu\text{mol/L}$ in non-icteric samples to 13.4–17.7 $\mu\text{mol/L}$ in slightly icteric samples with I-index 1+ to 2+, and the icteric interference further increased with increasing bilirubin levels in a dose-dependent manner. The Beckman Coulter SYNCHRON and AU assays, despite the aforementioned positive bias of around 40 and 60 $\mu\text{mol/L}$, respectively, were relatively unaffected at this range of total bilirubin level, yet still positively interfered with higher bilirubin concentrations. The Sekisui and Siemens assays were relatively unaffected by icteric interference up to a mean total bilirubin of 537 $\mu\text{mol/L}$ (I-index 4+).

The icteric interference was less significant in samples containing a higher level of acetaminophen for most assays; however,

TABLE 2 Recovery of serially diluted samples at different calculated values of acetaminophen (difference from the calculated value for 0 $\mu\text{mol/L}$; percentage of recovery for 400–3200 $\mu\text{mol/L}$)

Assay	Calculated values ($\mu\text{mol/L}$)								
	0	400	800	1200	1600	2000	2400	2800	3200
AU	31.6	106.2%	108.2%	104.7%	105.3%	102.0%	101.2%	102.3%	99.2%
MULTIGENT	0.5	95.5%	100.7%	99.4%	100.1%	98.1%	97.3%	98.5%	95.8%
L3K	−3.0	102.5%	104.9%	105.8%	106.8%	103.9%	103.5%	103.5%	101.6%
ACTM	52.3	139.5%	131.3%	133.3%	122.6%	108.6%	96.8%	87.7%	79.1%
SYVA	0.0	101.0%	102.4%	100.4%	102.4%	102.1%	101.0%	97.3%	90.1%

ACTM, Beckman Coulter SYNCHRON Systems ACTM; AU, Beckman Coulter AU Paracetamol; L3K, Sekisui Acetaminophen L3K; MULTIGENT, Abbott MULTIGENT Acetaminophen; SYVA, Siemens SYVA Emit-tox Acetaminophen

TABLE 3 Summary of interferences on the five acetaminophen assays

Assay	Difference in value (for acetaminophen level of 0 and 100 $\mu\text{mol/L}$) or deviation in % (for acetaminophen level of 1000 $\mu\text{mol/L}$)						
	Intralipid	Hemoglobin	Bilirubin			NAC	
Interference	0-11.3 (mmol/L)	0-10.0 (g/L)	Normal-537 ($\mu\text{mol/L}$)	11-517 ($\mu\text{mol/L}$)	10.5-451 ($\mu\text{mol/L}$)	0-12.3 (mmol/L)	0-12.3 (mmol/L)
Acetaminophen ($\mu\text{mol/L}$)	100	100	0	100	1000	100	1000
AU	-5.7 to -23.1	12.2 to 62.5	-0.3 to 28.9	-20.8 to -21.5	-4.2 to -15.2	-13.6 to -43.4	-12.8 to -36.5
MULTIGENT	-4.3 to -13.5	2.4 to 53.4	15.7 to 86.7	37.5 to 81.7	-0.6 to -2.3	-52.4 to -84.1	-59.8 to -92.5
L3K	3.0 to 19.0	3.0 to 43.0	-2.0 to 11.0	-10.0 to -2.0	-3.6 to -5.0	-1.0 to 1.0	-0.7 to -0.9
ACTM	32.6 to 144.0	-3.0 to -9.1	3.6 to 18.2	14.9 to -2.3	-7.9 to -7.6	-3.2 to -14.3	-0.8 to -1.5
SYVA	-3.0 to -7.0	-5.0 to -3.0	1.0 to 0.0	2.0 to 6.0	0.1 to -5.9	0.0 to -7.0	5.0 to 1.9

ACTM, Beckman Coulter SYNCHRON Systems ACTM; AU, Beckman Coulter AU Paracetamol; L3K, Sekisui Acetaminophen L3K; MULTIGENT, Abbott MULTIGENT Acetaminophen; SYVA, Siemens SYVA Emit-tox Acetaminophen.

the Abbott assay still showed a dose-dependent positive icteric interference at low acetaminophen level (100 $\mu\text{mol/L}$), while at high acetaminophen level (1000 $\mu\text{mol/L}$) such interference became insignificant. On the other hand, on top of the positive bias, a negative icteric interference was demonstrated in the Beckman Coulter AU assay, which was more significant at high I-index of 3 + and 4 + or above at both low and high acetaminophen levels, respectively. This negative icteric interference was also observed in Beckman Coulter SYNCHRON assay at high acetaminophen levels (also on top of the positive bias), but to a lesser extent. The results of icteric interference study of the five acetaminophen assays on samples with different acetaminophen levels are summarized in Table 3.

3.4.4 | NAC interference

Both immunoassay-based assays and the Sekisui assay were relatively unaffected by NAC. Both the Abbott assay and the Beckman Coulter AU assay showed dose-dependent negative interference by the addition of NAC, at both high and low concentrations of acetaminophen. The magnitude of negative interference of NAC on acetaminophen measurement was most significant in the Abbott assay where twofold to tenfold decrease in measured acetaminophen level for sample spiked with 3.06-12.3 mmol/L NAC was observed. The results are summarized in Table 3.

3.4.5 | Interference due to sample matrix

It was noted that Beckman Coulter SYNCHRON and AU assays produced significantly higher acetaminophen results on blank samples (ranging 45.4-63.2 $\mu\text{mol/L}$ for SYNCHRON and 33.0-37.6 $\mu\text{mol/L}$ for AU) than those obtained from other assays. For the AU assay, effect of protein matrix was demonstrated with a good correlation between the total protein concentrations and apparent acetaminophen concentrations measured in these samples ($R^2=0.9984$).

3.5 | Recovery of acetaminophen after ultrafiltration

Enzymatic acetaminophen assays are prone to icteric interference, and ultrafiltration is considered one of the ways to eliminate such interference. Recovery of acetaminophen in the ultrafiltrate was estimated with the three enzymatic methods at acetaminophen levels of 99, 331, and 992 $\mu\text{mol/L}$. The Abbott and Sekisui assays showed superior performance (with recovery of $89.5\% \pm 3.0\%$ and $88.0\% \pm 2.7\%$, respectively) to the Beckman Coulter AU assay (with $80.0\% \pm 5.1\%$). The results of post-ultrafiltration recovery are summarized in Table 4.

4 | DISCUSSION

In the current study, the analytical performances of the enzymatic acetaminophen assays are generally superior to the immunoassay-based assays in terms of precision, accuracy, and linearity ranges. The three enzymatic assays have better within-run imprecision ($<1\%$) and between-run imprecision ($<3\%$) over a wide range of acetaminophen levels. The immunoassay-based methods have significantly larger within-run and between-run imprecisions. The Beckman Coulter SYNCHRON assay showed significant high bias beyond the APS. This high bias was unlikely due to calibration error, as we detected no discrepancy in the measured values and the assigned values of the SYNCHRON assay's calibrators when assayed with other analytical platforms.

For acute acetaminophen toxicity, the serum levels are usually grossly elevated; however, for delayed presentation, for example, at around 24 hour post-ingestion, the serum acetaminophen can be as low as 20 $\mu\text{mol/L}$, yet still above the treatment line in the Rumack-Matthew nomogram.⁸ Despite satisfactory EQA performance, the Beckman Coulter AU showed high background signals on control blank serum samples with apparent acetaminophen

TABLE 4 Recovery after ultrafiltration for the three enzymatic assays

Acetaminophen assay	99 $\mu\text{mol/L}$	331 $\mu\text{mol/L}$	992 $\mu\text{mol/L}$
Beckman Coulter AU paracetamol	74.6%	80.5%	84.7%
Abbott MULTIGENT acetaminophen	92.8%	87.2%	88.5%
Sekisui acetaminophen L3K	90.5%	88.6%	85.2%

levels of $35.3 \pm 1.7 \mu\text{mol/L}$ (mean $\pm$ SD) in control blank, which was beyond the claimed linearity. This was probably caused by matrix interference due to chemical reaction between the Cu^{2+} and proteins present in samples. In fact, Cu^{2+} was used in Biuret reaction and Folin-Ciocalteu reagent for protein measurements. A positive correlation between the total protein concentrations and apparent acetaminophen concentrations was observed at blank serum and the low end of the measuring range. Similar pattern was observed in the Beckman Coulter SYNCHRON assay. In the Abbott and Sekisui assays, Cu^{2+} was replaced by Mn^{2+} so as to eliminate the protein interference and there were no significant background signals at blank or high bias at low end observed.

The enzymatic assays had wider linearity ranges than the immunoassay-based assays: The Beckman Coulter AU, Abbott, and Sekisui assays showed linearity to acetaminophen level of more than $3000 \mu\text{mol/L}$, while the linearity range of the Beckman Coulter SYNCHRON and Siemens assays was up to around $1600 \mu\text{mol/L}$ (which was lower than the claimed $1986 \mu\text{mol/L}$) and $2425 \mu\text{mol/L}$, respectively.

The enzymatic assays are generally more susceptible to various endogenous and exogenous interferences, especially bilirubin and hemoglobin, when compared to the immunoassay-based assays.⁴ All of the three enzymatic assays were shown to tolerate low level of hemolytic interference (up to hemoglobin level of 2 g/L), while beyond this level a significant positive interference was demonstrated.

Bilirubin causes interferences in acetaminophen measurements with its broad and intense absorbance in the ultraviolet and visible regions of the electromagnetic spectrum, as well as the potential reactions with the reagents of the enzymatic assays by its metabolites or itself (ie, chemical interference). Among the three enzymatic assays evaluated in the study, the Abbott assay was most susceptible to icteric interference. The Beckman Coulter AU assay could tolerate mildly icteric sample, while in moderate-to-severe icteric samples positive interference at blank sample could be observed; paradoxically, these levels of bilirubin would cause negative interference in samples containing acetaminophen. These false-positive or false-negative results could potentially lead to inappropriate treatment decisions in patient with exposure to acetaminophen or even misdiagnosis. Apart from the hemolytic and icteric interferences, the Abbott and Beckman Coulter AU assays (especially the former) were

also subjected to significant negative interference in the presence of therapeutic concentrations of NAC.

To correct for bilirubin interference, mathematical correction based on the results obtained from samples spiked with unconjugated bilirubin was suggested but was not commonly used.⁶ Ultrafiltration has been used in clinical laboratories for eliminating bilirubin interference.⁴ In the current study, samples (with bilirubin concentration greater than which interference would occur) were subjected to ultrafiltration before acetaminophen measurement. The Abbott assay demonstrated satisfactory recovery, while the Beckman Coulter AU assay had a generally lower recovery.

The Sekisui assay was demonstrated to have the best analytical performance among the three enzymatic assays, especially in interference studies. It was generally unaffected by NAC. Mild positive interference by total bilirubin was demonstrated in blank samples but not at higher acetaminophen levels. While the claimed limit of quantitation of $4 \mu\text{mol/L}$ could be verified in blank samples, there was significant icterus interference at low end of the analytical range, and thus, the lowest reporting limit should be set at a higher level (eg, $30 \mu\text{mol/L}$), when such a level is still adequately low for clinical use.

In contrast to the enzymatic acetaminophen assays, the immunoassay-based assays are generally less susceptible to hemolytic, icteric, and NAC interferences, with also better reagent stability.

Among the two immunoassay-based assays, the Siemens SYVA Emit-tox Acetaminophen assay has the better performance in terms of accuracy, linearity, and tolerant to interference. The disadvantages include the slightly inferior performance in precision (and thus EQA performance), higher per-test cost, more calibration points required, and yet a narrower dynamic range. Mass spectrometric methods for acetaminophen measurement would have the highest specificity when free from common interference,⁹ which may, however, be unsuitable for a 24-hour clinical emergency service.

In conclusion, among the five acetaminophen assays evaluated, all had satisfactory accuracy as reflected by EQA performance, except the Beckman Coulter SYNCHRON assay, in which bias and significant lipemic interference were both observed. The enzymatic assays overall had better analytical performances in terms of accuracy, precisions and linearity range: Both the Abbott and the Beckman Coulter AU assays were susceptible to hemolytic, icteric, and NAC interferences, which might be partially eliminated with sample ultrafiltration. The Sekisui assay shared the advantages of other enzymatic methods of being a simple, efficient, robust, and relatively inexpensive automated assay, which is more specific and less affected by the aforementioned interferences despite the icteric interference at low end. The Siemens assay is virtually free from bilirubin, hemoglobin, lipids, and NAC interferences, with a much lower limit of quantitation than the Sekisui assay, but still it may be susceptible to the disadvantages of an immunoassay-based method.

Different reagent manufacturers developed and launched their acetaminophen assays with modified formulations and advance technologies in the recent decade. Besides, improvement in analytical performance of automated chemistry analyzers is also expected.

However, disappointing analytical performance of existing reagent on our analyzer was observed. With the opportunity seeking for alternatives and availability of resources, the study provides comprehensive and updated information on five selected commercial acetaminophen assays on our analytical platform.

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CONFLICT OF INTEREST

None.

ORCID

Hing-Man Tsang <http://orcid.org/0000-0002-6452-7691>

Hencher Han-Chih Lee <http://orcid.org/0000-0002-0162-5681>

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