Analytical performance of a compact veterinary chemistry analyzer (PT10V) at multiple local clinics

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Key Words: Samsung PT10V analyzer, POC, Dry chemistry, Canine, Feline, Total error

Background: PT10V is a newly introduced point-of-care (POC) dry chemistry analyzer that uses a thin film-based cartridge technology for canine, feline, and equine blood samples. No previous published reports have evaluated the analytical performance of this device.

Objectives: The objectives are to (1) validate the analytical performance of PT10V using canine and feline serum samples and compare its performance with a reference analyzer (Hitachi 7020), (2) compare its performance with other three commercialized POC analyzers.

Methods: Thirteen analytes were measured using serum from dogs and cats. Observed total error (TE_o) was calculated based on coefficient of variation (CV) and bias. TE_o was compared with allowable total error (TE_a). Canine and feline samples were collected and measured in five small clinical sites and one reference site. Passing-Bablok regression and Bland-Altman analysis were used to compare PT10V with the reference analyzer and the other POC analyzers.

Results: For all analytes, TE_o calculated by using quality control material (QCM)-based CV and bias met ASVCP-recommended TE_a. PT10V and the reference analyzer had high correlation coefficients ($r > 0.70$) for 12/13 (92%) of analytes in dogs, and 13/13 (100%) of analytes in cats. Among the clinical sites, there was no significant difference in PT10V's performance for the analytes having similar sample distributions for each site. The correlations among four POC analyzers including PT10V compared to the reference were similar to each other for all analytes in dogs and cats.

Conclusions: The analytical performance of PT10V is "acceptable" compared with the reference analyzer, and "comparable" to the other routine POC analyzers. PT10V is useful in routine blood chemistry testing in general veterinary clinics.

Introduction

The Samsung PT10V chemistry analyzer (hereafter referred to as PT10V; Samsung Electronics, Suwon, Korea) is introduced recently, and adopts a compact and easy-to-use cartridge and system to measure concentrations of various serum or plasma constituents (19 analytes) by colorimetric, end-point, kinetic, and ion-selective optode methods.[1] Like other chemistry analyzers used in private veterinary clinics, PT10V has several advantages with rapid results (less than 7 min), the need for small sample volume (about 70 μl), and the built-in internal quality control program, which will be discussed in next article. A thin film-based cartridge has 16 wells with dry reagents for corresponding analytes. So, the sufficient sample volume per analyte is less than 5 μl.

The objectives of this article are to evaluate the performance of PT10V, compare its performance with the reference instrument and the other three commercialized POC analyzers, and evaluate its potential for use by practicing veterinarians.

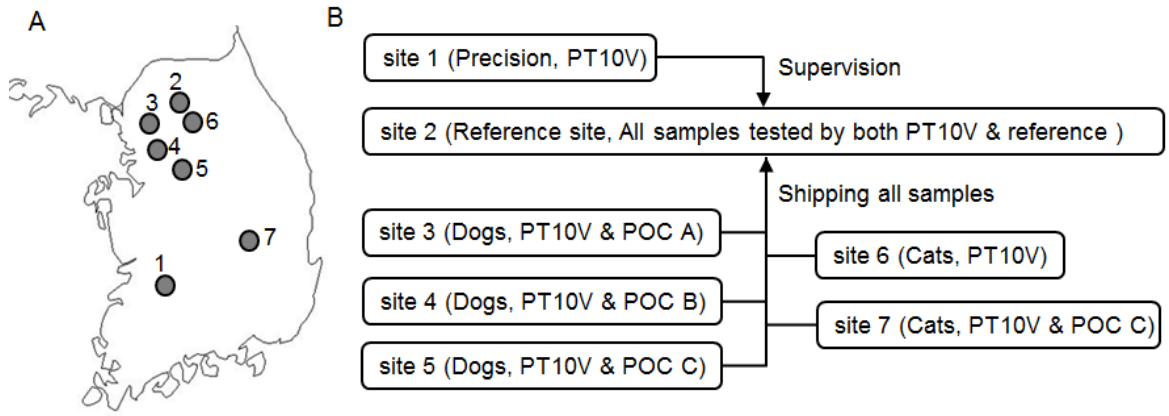

Figure 1. Experimental design. (A) Locations of 7 different clinical sites. (B) One site is a supervisor, another is a reference site to measure both reference and PT10V's values for all clinical samples (dogs, cats), and the others are small clinics to measure PT10V and other POC's values right after sample collection.

Material and Methods

Equipment and cartridge

PT10V is a blood chemistry analyzer designed to test animal plasma and serum samples simultaneously for up to 15 analytes at a time. It has an electrical quality control and other self-diagnosis modes, so that users can easily check and monitor the instrument. It requires little maintenance because the manufacturer performs calibration for each lot and users only need to run external quality control using QCM for quality management. In addition, PT10V can be integrated into a hospital or laboratory information system (LIS).*[1] The sample moves to the dry reagents for reaction and the reaction is monitored at a particular wavelength to determine quantitative results. A 70-µl blood sample is inserted into the PT10V cartridge and the analyzer provides multi-parameter testing of up to 15 analytes within 7 min.

In this study, PT comprehensive Test 13V cartridge was used to measure thirteen analytes: albumin (ALB), alkaline phosphatase (ALP), alaline amino-transferase (ALT), amylase (AMY), blood urea nitrogen (BUN), calcium (CA), cholesterol (CHOL), creatinine (CREA), glucose (GLU), phosphate (PHOS), total bilirubin (TBIL), total protein (TP), and triglycerides (TG).

Study design

Overall study design in multiple veterinary clinics is shown in Figure 1. The site 1 (Chunbuk National Uni., Korea) supervised all the study and conducted the precision analysis of PT10V by using QCM. The target number of blood samples were 170 (dogs) and 80 (cats). Most of the samples were collected in 5 different small clinics (site 3~7) and the rest of the samples were collected in the reference site (site 2) to make up a wide range of sample distribution and provide more accurate evaluation of the analytical performance between PT10V and the reference instrument (Hitachi 7020). The small clinics (site 3~7) were selected by the capability of species-specific sample collection (site 3~5: dogs, site 6~7: cats) and their own POC analyzer*[2] that was routinely used in house. From this multi-site study design, PT10V was evaluated for in-situ usability and site-by-site variability.

Samples

Collected individual patient samples were processed via standard laboratory protocol in each site. Following accession by the protocol, samples were allowed to clot by leaving it undisturbed at room temperature, and the clot was removed by centrifuging at 1,000~2,000 g for 10 min.

For processed samples, PT10V provides serum indices (hemolytic, HEM; icteric, ICT; lipemic, LIP) to prevent one of the main pre-analytical errors, *i.e.*, the interference caused by sample integrity. In Table 1, the grade of three serum indices is shown as 0 (none), +1 (slight), +2 (moderate), and +3 (severe).

*[1]PT10V provides standard ASTM, HL7, and POCT1-A interfaces.

*[2]POC A and POC C are slide-type dry chemistry analyzers, and POC B is a rotor-type chemistry analyzer.

Table 1. Serum indices of hemolysis, icterus and lipemia in PT10V (See Glossary terms)

Serum Index			Hemolysis	Icterus	Lipemia
			mg/dL	mg/dL	mg/dL
Grade	0	None	< 60	< 1.5	< 180
	+1	Slight	60-120	1.5-4.1	180-350
	+2	Moderate	121-150	4.2-9.0	351-900
	+3	Severe	> 150	> 9.0	> 900

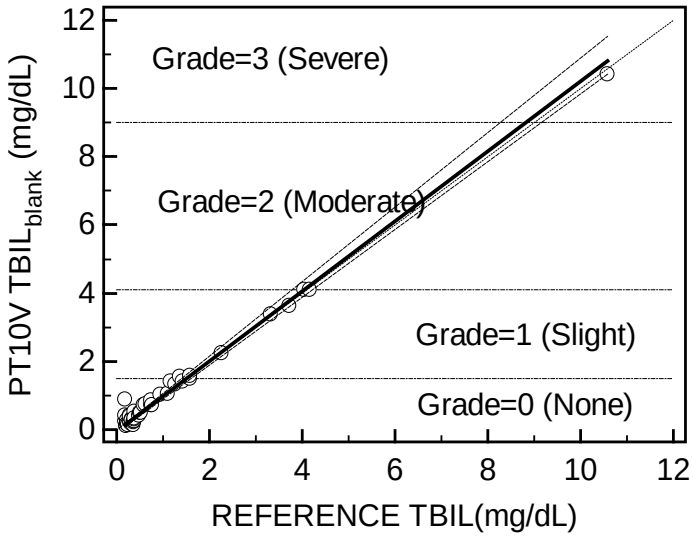

Figure 2. Validation of icteric index (ICT) compared to a reference (Cobas p-modular; Roche) TBIL value. TBIL$_{blank}$ was calculated by the patented algorithm based on a serum blank in the cartridge.

On the basis of the index grade, hemolytic samples over grade +1 (slight) and lipemic samples over grade +1 were excluded.

Figure 2 shows the icteric index (ICT) as an example to validate the serum indices. ICT was compared with a reference TBIL assay (Cobas P-modular, Roche) and calculated by colorimetric intensities from multiple optical wavelengths at a serum blank in the cartridge. Its resolution is highly close to the reference TBIL assay.

Precision and total error

Precision testing was performed on PT10V by using commercially available human serum-based QCM with 2 different levels (Accusera; Randox Lab., UK). QCM was run twice per day during five days (CLSI EP5-A3). Each test was performed within 10 min.

Observed total error of PT10V was calculated according to the formula[2] of

$$TE_o(\%) = \text{bias} + 2CV$$

The bias was measured for each analyte using QCM and the target was the middle of the manufacturer's reported range for the analyte. The CV was total imprecision including within-run, between-run, and between-day CVs.

Method comparison

Results from PT10V were compared with those from the reference instrument (Hitachi 7020) and three other POC analyzers (POC A~C) using Passing-Bablok regression, Bland-Altman bias analysis and Pearson correlation analysis. In the regression analysis, the cumulative sum (cusum) linearity test was performed to investigate possible significant deviation from linearity between two sets of data.[3] By the Kolmogorov-Smirnov test, normality of sample distribution was also checked.

For method comparison, PT10V was run once per sample. This single-run test protocol has been recently used as in the evaluation of POC analyzers and adopted in this study to mimic real in-house use in a local veterinary clinic.

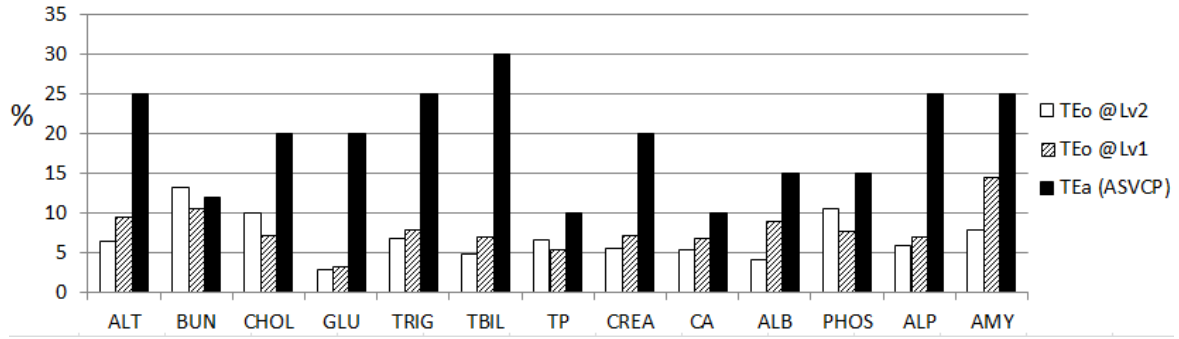

Figure 3. Comparison of observed total error (TE_o) and allowable total error (TE_a) recommended by ASVCP for each analyte. TE_o based on QCM was calculated for low (Lv 1) and high concentration (Lv 2).

Results and Discussion

Precision study

The total imprecisions (CV) at QCM's level 1 and 2 for all analytes were within 5% except for BUN, PHOS, and AMY. The total CV for BUN was 5.2% at level 1 and 6.6% at level 2; for PHOS was 5.3% at level 2; and for AMY was 7.2% at level 1.

In Figure 3, with the total CVs and the biases based on QCM, the observed total error (TE_o) was calculated and compared to the allowable total error (TE_a) recommended by ASVCP*[3] for each analyte.[4] Using this TE_o, all PT10V analytes met the chosen quality requirement ($TE_o \leq TE_a$).

Method comparison study

For PT10V and reference instrument (canine & feline samples)

The interpretation method of correlation coefficients was the same as Flatland *et al*'s: r = 0.90−1.0 defined by very high correlation; 0.70−0.89, high correlation; 0.50−0.69, moderate correlation; 0.30−0.49, low correlation.[2]

Data for canine samples between PT10V and the reference instrument are summarized in Table 2 *(in page 5)* and graphically shown in Figure 5 (*in page 6*). 12 of 13 (92%) analytes have very high (9/13, 69%) and high (3/13, 23%) correlation. The only calcium (CA) has moderate correlation as r = 0.67. The relatively lower correlation was due to two main reasons: (i) restriction of sample distribution, (ii) different methodology in reagent assays between two instruments. Compared to the measuring range of CA, the canine samples were almost normal and distributed within 25% of the measuring range. For calcium assay, Hitachi 7020 uses the o-cresolphthalein method, whereas PT10V uses the Arsenazo III method. When PT10V's CA was compared to POC B's (in site 4) as evidence, the correlation coefficient of CA became higher as r = 0.80. The reason is why POC B's CA method is the same as PT10V's.

Data for feline samples between PT10V and the reference instrument are summarized in Table 3 (*in page 5*) and graphically shown in Figure 6 (*in page 6*). All of 13 analytes have very high (9/13, 69%) and high (4/13, 31%) correlation.

The best approach to measure systematic bias has been debated in laboratory medicine and entails two main difficulties. The first is that bias is relative, depending on the measure used to define the truth. The second difficulty is that bias assessment is complicated by the issue of species specificity. In this study, the site 2 as a reference site used Hitachi 7020 with its own *de novo* reference intervals in various veterinary species.[5] In addition, PT10V had the traceability with Cobas Modular Series (Roche) as a reference. Therefore, relatively large systematic biases can occur between PT10V and Hitachi 7020. The bias will be re-evaluated using a different reference instrument with the same traceability as PT10V's.

For PT10V and reference instrument (site-by-site)

In Figure 4, correlation coefficients (r) were compared site-by-site for 7 analytes, which satisfied the selection criterion of similar sample distribution.

*[3]ASVCP: American Society of Veterinary Clinical Pathology

Table 2. Regression and bias analysis for canine samples measured by PT10V (x-axis) and Hitachi 7020 (y-axis).

Canine Specimens		Correlation	Passing-Bablok Regression				Bland-Altman Plot	
Analyte	n†	R	y-intercept	95% CI	Slope	95% CI	Mean difference	LOA*
ALB	172	**0.76**	**-2.53**	-3.60 to -2.00	**1.67**	1.50 to 2.00	**0.44**	-0.13 to 1.01
ALP	167	**0.99**	**-16.40**	-19.61 to -13.59	**1.45**	1.41 to 1.50	**-53.5**	-217.6 to 110.5
ALT	170	**0.97**	**-6.17**	-7.94 to -4.43	**0.66**	0.63 to 0.69	**39.2**	-30.8 to 109.2
AMY	171	**0.96**	**26.12**	-15.50 to 64.73	**1.22**	1.17 to 1.27	**-213.8**	-525.9 to 98.3
BUN	164	**0.99**	**3.21**	2.71 to 3.75	**0.80**	0.77 to 0.82	**1.5**	-7.4 to 10.4
CA	173	**0.67**	**-8.24**	-11.00 to 5.85	**1.73**	1.50 to 2.00	**0.76**	-0.96 to 2.48
CHOL	170	**0.97**	**6.52**	1.58 to 10.15	**0.62**	0.60 to 0.65	**76.8**	15.1 to 138.4
CREA	174	**0.96**	**0.16**	0.10 to 0.22	**0.78**	0.71 to 0.86	**0.05**	-0.52 to 0.61
GLU	172	**0.96**	**8.55**	2.73 to 12.85	**1.01**	0.96 to 1.07	**-9.1**	-29.2 to 11.0
PHOS	174	**0.88**	**-0.60**	-0.99 to -0.20	**1.16**	1.07 to 1.27	**-0.1**	-2.1 to 1.8
TBIL	119‡	**0.98**	**N/A**	N/A**	**N/A**	N/A	**-0.03**	-0.34 to 0.29
TP	173	**0.74**	**-0.71**	-1.65 to 0.20	**1.14**	1.00 to 1.28	**-0.17**	-1.26 to 0.91
TG	174	**0.95**	**6.72**	1.88 to 11.66	**1.37**	1.29 to 1.47	**-34.4**	-85.8 to 17.1

*LOA = limits of agreement (+/-1.96 standard deviations from the mean difference).
**N/A = not applicable due to significant deviation from linearity ($p<0.01$).

†Target number for canine sample is 170. For n>170, samples were collected and tested more to make up insufficient sample distribution, and, for n<170, the excluded data were out of reportable range.

‡TBIL values of 30% were out of the lower limit of reportable range (<0.1 mg/dL).

Table 3. Regression and bias analysis for feline samples measured by PT10V (x-axis) and Hitachi 7020 (y-axis).

Feline Specimens		Correlation	Passing-Bablok Regression				Bland-Altman Plot	
Analyte	n†	R	y-intercept	95% CI	Slope	95% CI	Mean difference	LOA*
ALB	82	**0.81**	**-2.30**	-2.90 to -1.10	**1.33**	1.00 to 1.50	**1.15**	0.72 to 1.59
ALP	85	**0.99**	**-18.52**	-21.56 to -15.14	**1.67**	1.57 to 1.75	**-14.0**	-96.5 to 68.5
ALT	85	**0.89**	**-13.86**	-18.00 to -8.73	**0.93**	0.84 to 1.00	**16.2**	-12.9 to 45.4
AMY	81	**0.95**	**-66.56**	-173.1 to 31.0	**1.36**	1.27 to 1.47	**-364.2**	-913.9 to 185.5
BUN	80	**1.00**	**2.48**	1.47 to 3.61	**1.01**	0.95 to 1.05	**-3.0**	-7.6 to 1.7
CA	83	**0.70**	**-5.95**	-10.70 to -2.42	**1.54**	1.20 to 2.00	**0.53**	-1.23 to 2.29
CHOL	85	**0.98**	**2.67**	-1.28 to 6.30	**0.67**	0.65 to 0.70	**50.9**	2.2 to 99.6
CREA	85	**0.91**	**0.35**	0.21 to 0.44	**0.65**	0.60 to 0.72	**0.4**	-1.1 to 1.8
GLU	82	**0.95**	**9.10**	1.04 to 15.9	**1.03**	0.98 to 1.09	**-12.0**	-38.6 to 14.5
PHOS	85	**0.94**	**-0.52**	-1.26 to 0.20	**1.13**	1.00 to 1.30	**-0.2**	-2.3 to 1.9
TBIL	26‡	**0.99**	**-0.07**	-0.35 to 0.00	**1.56**	1.18 to 3.33	**-0.19**	-0.79 to 0.40
TP	85	**0.84**	**-0.55**	-1.69 to 0.41	**1.07**	0.94 to 1.23	**0.01**	-1.14 to 1.16
TG	84	**0.97**	**-2.71**	-10.74 to 4.32	**1.32**	1.21 to 1.44	**-20.4**	-74.5 to 33.6

*LOA = limits of agreement (+/-1.96 standard deviations from the mean difference).

†Target number for canine sample is 80. The number difference has the same reason as canine samples.

‡TBIL values of 67% were out of the lower limit of reportable range (<0.1 mg/dL).

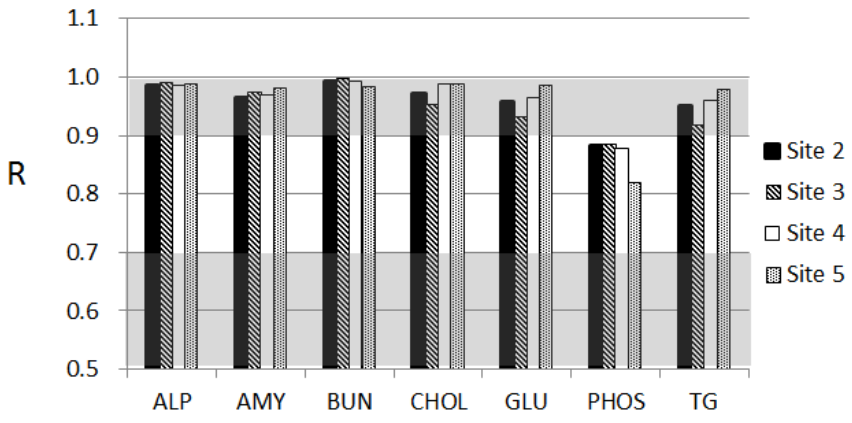

Figure 4. Comparison of correlation coefficients among 4 clinical sites for canine samples. 7 of 13 analytes were selected with respect to similar sample distributions. The number of samples in site 2 was the same as in Table 2, and the number of samples in site 3~5 is 50 per each site.

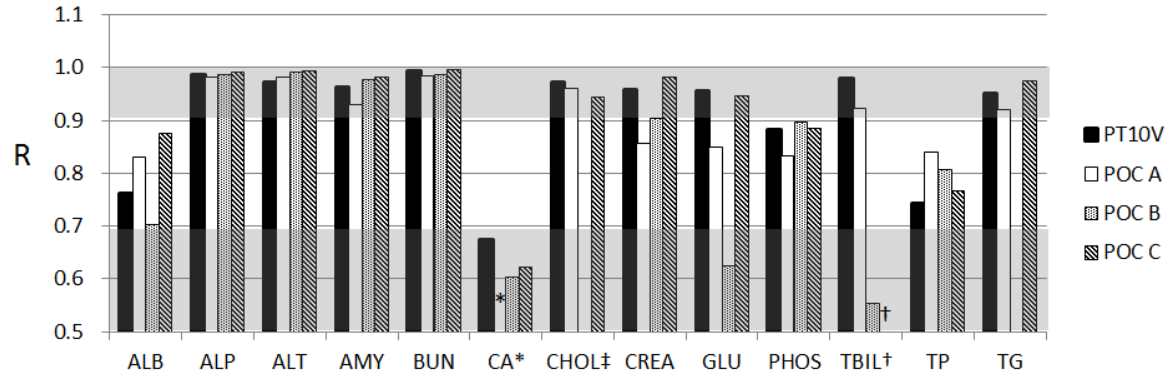

Figure 5. Comparison of correlation coefficients among 4 POC analyzers for canine samples. *CA for POC A was not shown due to its low value (0.13). ‡CHOL for POC B was not tested. †The correlation coefficient of TBIL for POC C was a negative value and not available due to highly scattered data.

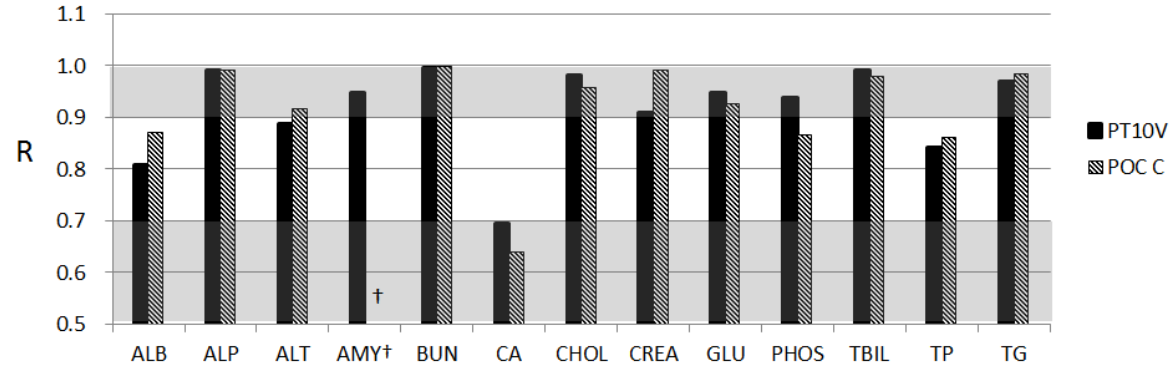

Figure 6. Comparison of correlation coefficients among 2 POC analyzers for feline samples. †AMY for POC C was not tested.

The results show that the correlation coefficients varied within 10% among sites. This means that PT10V's performance was not affected by various test environments including different operators and geographic regions.

For PT10V and other POC analyzers

One of our main objectives was to validate the comparability to the other POC veterinary analyzers. Figure 5 and 6 shows the results to compare the correlation coefficients among four POC analyzers including PT10V. For both canine and feline samples, there was no significant difference among POC analyzers even though their methodologies were a little different.

Conclusion

PT10V has a high precision and satisfies the recommended quality requirement ($TE_o \leq TE_a$). In the study of multiple clinical sites, it shows the robustness for various test conditions and ensures the high comparability over the other commercialized veterinary chemistry analyzers. In consideration of this analytical performance, PT10V can be a reliable instrument for general veterinary practice in routine in-clinic use.

References

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Glossary Terms

Statistical Terms

TE (total error, total analytical error): The sum of random error (imprecision) and systematic error (bias or inaccuracy). This term may also incorporate other sources of error (e.g. some pre-analytical variation, biological variation, and other factors) that contribute to variation seen in patient results. Total error components that are under direct supervision or control of the laboratory are bias and imprecision.

TE_a (allowable or desirable total error): A quality requirement that sets a limit for combined imprecision (random error) and bias (inaccuracy, or systematic error) that are tolerable in a single measurement or single test result to insure clinical usefulness.

TE_o (observed or calculated total error): The sum of measured random error (imprecision) and measured systemic error (bias or inaccuracy). TE_o is defined in this guideline as:

If expressed in units of %,

TE_o = 2CV + absolute bias%

If expressed in analyte units,

TE_o = 2SD + absolute mean difference

Bias (or Inaccuracy): Total systematic error, which includes constant and proportional bias. Bias is the difference between the measured result and some measure of the "true" value (e.g. as measured by a reference method or as defined by a known standard). The term *bias* has a specific meaning in the statistical t-test and in difference plot analysis, where bias (expressed in analyte units) equals the difference between the mean values of 2 methods being compared or the average of all the differences between the paired sample values. Bias may also be expressed as a percentage according to the formula

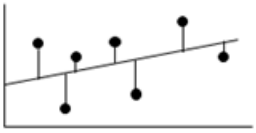
$$\text{Bias\%} = \frac{Mean_{target} - \text{Mean}_{\text{measured}}}{\text{Mean}_{\text{target}}} \times 100$$

Bias can be positive or negative; when used to calculate observed total error, the absolute value is used. Recommendations in this paper focus on using a known mean concentration of commercially available assayed control material as the target mean, since control materials are most easily accessible and cost-effective for privately practicing veterinarians. In clinical pathology laboratories, best practice dictates that target means be based on data from method comparison to a true reference method ("definitive" method) or known concentration of certified reference material. Target means may also be based on peer group means from external quality assessment (EQA, or proficiency testing) program data.

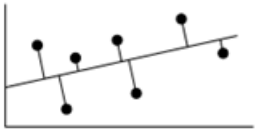
Coefficient of Variation (CV): A measurement of imprecision (random error), biologic variation, or other variability in a population; mathematically, CV is standard deviation (SD) divided by the mean (mathematical average) and expressed as a percentage:

$$\text{CV}(\%) = \frac{\text{SD}}{\text{Mean}} \times 100$$

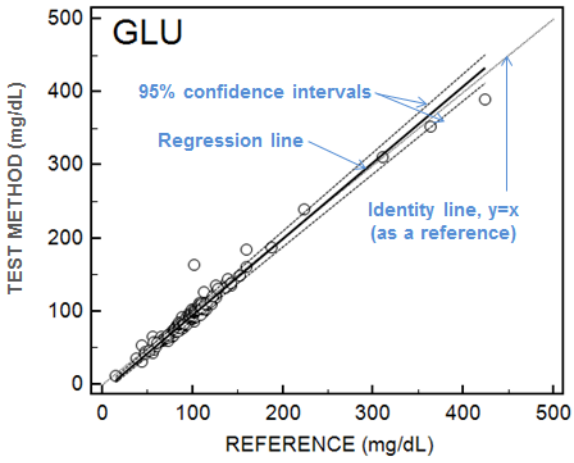
Passing-Bablok Regression: Passing & Bablok (1983) have described a linear regression procedure with no special assumptions regarding the distribution of the samples and the measurement errors. The result does not depend on the assignment of the methods (or instruments) to X and Y. The slope B and the intercept A are calculated with their 95% confidence interval. These confidence intervals are used to determine the limit of proportional bias from "1", and the limit of systematic bias from "0". This is different from the traditional ("least square") method which measures residuals parallel to the y-axis and is commonly used in Microsoft Excel program.



Least square regression (used in Microsoft Excel)

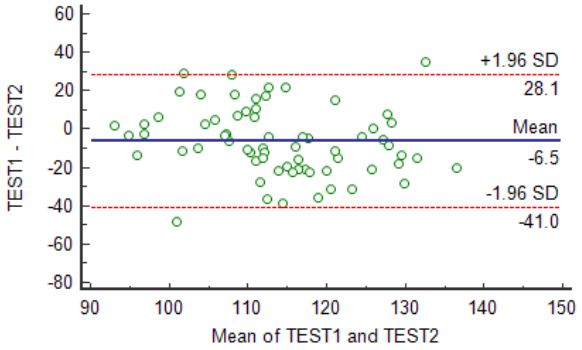


Passing-Bablok regression (Perpendicular residuals)



Bland-Altman Analysis (or Plot): The Bland-Altman plot, or difference plot, is a graphical method

to compare two measurements techniques. In this graphical method, the differences can be plotted against one of the two methods, if this method is a reference or "gold standard" method. Horizontal lines are drawn at the mean difference, and at the limit of agreement (LOA), which are defined as the mean difference plus and minus 1.96xSD of the differences.



Correlation Coefficient (R): The correlation coefficient is a number between -1 and +1. In general, it expresses the degree of strength and direction of the linear relationship between two variables. If one variable increases when the second one increases, then there is a positive correlation. In this case, the correlation coefficient will be closer to +1. If one variable decreases when the other variable increases, then there is a negative correlation and the coefficient will be closer to -1.
In this paper, the coefficient was interpreted by the same criteria recommended by Bente Flatland (DVM; Chair of QALS Point-of-Care Testing Committee). R=0.90-1.0 was defined by "very high" correlation, R=0.70-0.89 by "high" correlation, R=0.50-0.69 by "moderate" correlation, and R < 0.49 by "low" correlation.

Reference Interval (RI): The interval contains all the possible values between and including an upper and lower limit of normal healthy samples. Reference interval is preferred over the term "reference range."

***De novo* Reference Interval** : The "*de novo*" means "from the beginning'. The *de novo* RI is established by a specific laboratory from normal healthy samples.

Instrumental Terms

Reference Instrument (or Analyzer): The reference simply means "standard" or "gold standard" to have "true" values. Basically, the reference instrument is referred as the gold standard instrument to measure analytes very accurately by a routine quality management internally or externally.

External QC: QC procedures performed by laboratory or veterinary clinic staff that are external to (i.e. not built or programmed into) the laboratory instrument. Measuring quality control material (QCM) is a common example of external QC. Another material is a "standard" made by National Institute of Standards & Technology. This material's value can be considered as a "true" one, because the value is measured by the highest accurate method in the world.

Internal QC: QC functions that are internal to (i.e. built and programmed into) laboratory instruments and assess the analytical processes of those instruments.

Hitachi 7020 analyzer: The model Hitachi 7020 is a automatic wet-based clinical chemistry analyzer. It offers a performance of up to 36 test parameters simultaneously and up to 200 tests per hour by using a spectrophotometric method. It has been widely used in small and big laboratories in both human and veterinary clinics. Due to the high performance of Hitachi 7020, it can be used as a reference analyzer.

Cobas p-modular (Roche): The model provides the serum work area solution designed for high workload laboratories, enabling a throughput of 2,000 to 10,000 per day. It has been widely used in big laboratories in both human and veterinary clinics. Due to the high performance of Cobas p-modular, it can be used as a reference analyzer.

Pre-analytical Terms

Serum Index (or HIL index): The serum index is related to sample integrity, such as hemolysis, icterus, and lipemia/turbidity (HIL). Because of limited resources and budgetary constraints, the clinical laboratory relies on the manufacturer to document HIL estimates and interference claims in the product labeling. An automated HIL detection system offers an objective and consistent methodology for assessing sample quality. HIL indices are calculations based on absorbance measurements that provide quantitative estimates of hemolysis, icterus, and lipemia.

The CLSI guideline for HIL indices, C56-A, provides recommendations for:

- Establishing HIL indices to assess sample quality
- Establishing error flags for HIL interference
- Reporting interference effects of HIL in the reagent instructions for use (IFU).

This is an example of hemolyzed, icteric, and turbid samples with several grades;

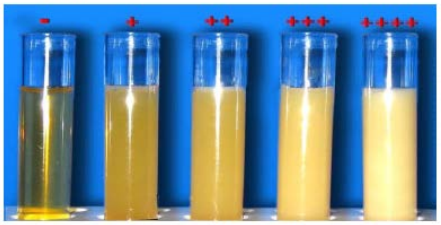
Hemolyzed (red)

mg/dL 0 50 150 250 525

Icteric (yellow)

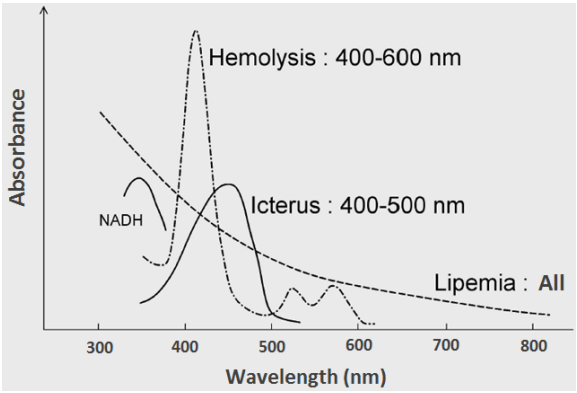
mg/dL 0 1.7 6.6 16 30

Lipemic (turbid)



mg/dL 0 125 250 500 1000

Hemoglobin, bilirubin, and lipids are the interference materials corresponding to the HIL indices. Those materials have unique spectral properties like:



Hemoglobin is red in color and absorbs light at wavelengths at 340-440 nm, and 540-580 nm. Bilirubin absorbs light strongly at 460 nm. The apparent absorption of light by lipemia is actually caused by the scatter of light by lipids and lipoprotein particles. The absorption is the highest below 400 nm and gradually decreases across the visible spectrum. There is still substantial light scattering at 700 nm.

Absorbance readings at several strategically selected wavelengths allow the calculation of the HIL indices. Instruments from different manufacturers vary in how they implement their HIL applications. Most instruments dilute the sample with physiological saline or buffer, but a few manufacturers take the absorbance readings on the neat sample.

Samsung PT10V adopted the direct measurement of absorbance for the neat sample, and implemented the patented algorithms to measure the HIL indices simultaneously while measuring chemistry analytes.