Applicability of Commercial Clinical Chemistry Test Kits for Horse Serum

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Research note

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Abstract

Objective: Validation of a test method is critical for confirming that the test can generate accurate and precise data. Although commercial biochemical test kits exist there is no specific and validated commercial clinical chemistry test kits designed for horse. The aim of this study was to validate commercial clinical chemistry test kits designed for human serum for use in horses.

Result: Blood samples were collected from 29 apparently healthy adult horses and pooled serum was prepared. Validation comprises replication and recovery experiments. Total observable error (TEo%), Sigma metrics and quality goal index (QGI) were used to support the validation studies. Intra- and inter-assay variability was 2.05% and 2.08%, 2.26% and 1.89%, 2.4% and 1.63%, for total cholesterol urea and total protein for respectively; recovery was 99.46%, 97.32% and 100.1% for total cholesterol urea and total protein for respectively. TE_o% for the specified analytes was within the allowable error. All the three anaytes satisfied the recommended requirement (>3 sigma values). The QGI for urea, as it had below 6σ was 0.95 indicating imprecision and inaccuracy. The results endorse the suitability of the studied commercial test kits and illustrated the acceptance criteria for serum collected from horse.

Introduction

Clinical laboratory plays a fundamental role in disease diagnosis, assessment of risk for a disease, monitoring to therapy and/or progression of a disease by providing timely data for patient management and disease surveillance [1]. One area in this regard is clinical chemistry laboratory and become popular in veterinary medicine [2]. It is indispensable that veterinary clinical laboratories must achieve accurate and precise test results. Laboratory tests performed using automated clinical chemistry analyser involves the instrument, the calibrator controls and one or more reagents [3]. Ensuring the consistency of the test results of a clinical laboratory before using reagents is vital to maintain that testing is done right and that it produces accurate results [4].

Quality in health care has immense impact on patient management as approximately 80% of all diagnosis is made on the basis of laboratory tests [5]. Method validation is one of the important quality system mechanisms that are designed to ensure the generation of scientifically valid and useful analytical data [6]. Although all commercial clinical chemistry kits are validated for their use in medical laboratories, they are commonly used also for other animal species [7]. It is imperative to conduct partial validation studies, independent of the manufacturer claim. Partial validation should be made to confirm the analytical procedure is fit for its intended purpose to be eligible it for use under actual settings [8]. According to the 2011 guideline on bioanalytical method validation, commercial kits need to be revalidated to ensure that the sample analysis is performed accurately and precisely. Additionally, a change of biological matrix or species is a reason to perform a partial validation, which can range from the determination of the within-run precision and accuracy to an almost full validation [9]. Validation is a pre-requisite to performing clinical sample assay and also key to satisfy regulatory requirement [10].

The clinical chemistry tests are often used for measurement of analytes. However, the quality of clinical chemistry may introduce systematic and random errors. This urges the need for validation of assay performance, regardless of its use in diagnosis or research [11]. Therefore the aim of the study is to validate commercially available kits for measurement of selected clinical chemistry in vitro diagnostic kits urea, total protein and total cholesterol designed for human serum for use in horse serum. The research was the first partial validation of the analytical performance of commercially available test kits in the veterinary clinical laboratory environment. Healthcare and veterinary professionals and academia in Ethiopia and elsewhere will benefit at large from the findings.

Materials And Methods

Study design

Study design was developed using American Society of Veterinary Clinical Pathology (ASVCP) guidelines: allowable total error guidelines for biochemistry [11]. Total allowable error for biochemical analytes was indicated in the guideline. Sample collection and animal use were approved by the institutional animal research ethics committee at the Addis Ababa university college of veterinary medicine and agriculture used in this study [Certificate reference no VM/ERC/09/01/12/2020].

Study animals, Sample size and sampling technique

The World Organisation for Animal Health (OIE) guideline 3.6.6 selection and use of reference samples and panels recommended minimum of 5 samples to prepare serum pool [12]. In addition to compute a statistically valid number of samples as suggested by Bayes Success-Run Theorem for validation studies 95% confidence and 90% reliability used. Therefore n = 28.4. We used 29 samples for the study [13]. Study animals were adult horses recruited by convenient sampling technique at society for protection of animal's abroad (SPANA-Ethiopia) clinic. Apparently healthy horses from owners who were consent after being informed about the purpose of the study were physically examined and blood sample was collected. Horses with history of medication excluded due to possible impact of drugs on analysis.

Collection and processing of blood samples

Blood samples from study animals were collected by a veterinarian from the jugular vein using standard operating procedure. The blood was allowed to clot at room temperature for between 30 minutes and serum was separated from the red blood cells by centrifugation at 1200xg for 10 minutes at 4 °C. Serum was immediately transferred to polypropylene tubes (Eppendorf Safe-Lock tubes) and stored at -20 °C until measurements. Samples were collected during two weeks in January 2020. Pooled serum samples were created by mixing equal volumes of individual serum then homogenized using an agitator for 10 min at 180 rpm. After homogenization aliquots of homogeneous pool was divided in to twenty portions to avoid effect of repeated thawing and freezing.

Analytical validation

To examine the accuracy and precision of a commercial clinical chemistry kits (Jourilabs diagnostics reagents and laboratory chemicals) for the quantifying concentration of total cholesterol, urea and total Protein was used and analysis of the parameters were determined by the methods/techniques described as follows: urea by kinetic urease/GLDH (Glutamate dehydrogenase), total protein by biuret and total cholesterol by CHOD-PAP (cholesterol peroxidase4-aminophenazone). The procedure of validation was adopted from Westgard JO method validation protocol. The analytical validation comprises of recovery studies for accuracy and replication experiments for precision [14]. All tests were performed on semi-automated chemistry analyser (AMP clinical Diagnostics, USA)

Replication experiments

Precision was assessed by evaluating the intra- and inter-assay variability using the pooled serum. Intra-assay variability (repeatability) was determined by measuring total cholesterol, urea and total protein in same sample 20 times sequentially within a single run. Inter-assay variability (reproducibility) was determined by analyzing the same sample in duplicate once on 20 consecutive working days. To avoid effect of repeated thawing and freezing, sample used for the determination of inter-assay variation were a liquated and stored at − 20 °C until use [15].

Recovery experiments

The Spike and recovery (SAR) assessment is essential for the analysis and accuracy evaluation of the method for particular sample types. Spike and recovery assay is used to determine whether the detection of an analyte is affected by biological sample matrix and differences in the standard curve diluent [16, 17]. Serum samples were spiked with different concentrations of standard Total cholesterol (26 mg/dl; 0.1 ml of 200 mg/dl standard solution was spiked in 1 ml serum) Urea (9.1 mg/dl; 0.1 ml of 100 mg/dl standard solution was spiked in 1 ml serum) and total protein (1.1 mg/dl; 0.1 ml of 12 mg/dl standard solution was spiked in 1 ml serum).

Quality requirement

Total Allowable Error (TE_a)

The analytical performance of the clinical chemistry parameters were assessed by calculating TE_{obs} (%) and σ values. TE_{obs} (%) was determined by the following formula: TE_{obs} (%) = 2 × CV + bias (%). Bias was calculated by the formula: Bias (%) = [(target − measured) ÷ target] × 100%, wherein “target” is the spiked value for each analyte and “measured” is the measured analyte concentration. TE_{obs} (%) was calculated using the inter-assay CV and bias (%). If TE_{obs} (%) is less than TE_a (%); the quality assessment passes and no further action needed. Criteria for acceptable performance or total allowable error TE_a (%) employed in this study (Total cholesterol: 20%, Urea: 12% and Total Protein: 10%) were adopted from American society of veterinary clinical pathology (ASVCP) guidelines: allowable total error guidelines for biochemistry [11].

Sigma metrics (σ)

Sigmas were calculated using the formula: σ = [TE_a (%) – bias (%)] ÷ CV. A method was considered acceptable if TE_{obs}< TEa. Interpretation of the σ values was performed as follows: >2: poor, > 3: marginal, > 4: good, > 5: excellent, and > 6: world class [18, 19].

Quality goal index ratio (QGI)

QGI ratio denotes the relative extent to which both precision and bias meet their respective quality. This was used to analyse the reason for the lower sigma in analytes, i.e., the problem is due to imprecision or inaccuracy or both. The QGI ratio was calculated as, QGI = Bias/1.5 × CV%. The criteria for interpreting QGI of the problem analytes with low sigma performance is as follows: QGI less than 0.8 shows imprecision, QGI falling in the range of 0.8 to1.2 shows both imprecision and inaccuracy and QGI greater 1.2 depicts inaccuracy [20].

Data analysis

Statistical analyses were performed using IBM SPSS 20. Normality distribution of the data was tested using the Kolmogorov–Sminorv test prior to statistical analysis. Data of accuracy from bias and precision from intra- assay and inter-assay CVs were estimated using routine descriptive statistical procedures.

Results

The present study analysed Total cholesterol, Urea and Total Protein from the pooled serum collected from 25 apparently healthy horses. The analysis was run on EMP-168 biochemical analyser Chengdu Empsun Medical Technology Co., Ltd

Precision was done by repeated measurements of pooled serum under specific and identical conditions on same day (intra-assay). For inter-assay repeatability, the pooled serum as frozen in separate vials at -20^{o}C, thawed at room temperature and assayed on 20 separate days. The data generated was calculated in terms of mean SD and CV is presented on Table1.

Table 1
Precision of the Pooled serum for Total cholesterol, Urea and Total Protein

Precision	**Intra-assay (N = 20)**			**Inter-assay (N = 20)**		
Parameters	Mean	SD	CV	Mean	SD	CV
Total cholesterol	80.3	1.65	2.05	80.4	1.67	2.08
Urea	80.3	1.82	2.26	80.4	1.52	1.89
Total Protein	6.25	0.15	2.4	6.13	0.1	1.63

To assess accuracy, a recovery method based on standard addition was used to evaluate the ability of the assay to recover the amount of analyte added to baseline pooled serum. The baseline pooled serum was obtained by the dilution of pooled serum with distilled water. While the spiking was done by addition of standard solutions to pooled serum then both diluted and spiked pooled serum was assayed on 5 replicates and the average value is depicted in Table 2.

Table 2
Recovery for the Pooled serum for Total cholesterol, Urea and Total Protein

Analyte	Addition	Dilution	Observed	Expected	Recovery
Total cholesterol	93.6	75.5	18.1	18.2	99.46%
Urea	66.72	64.7	8.86	9.1	97.32%
Total Protein	7.49	6.38	1.11	1.1	100.1%

The total observed error ($TE_{o)}$ assessed was expresses by combining random error (%CV) from the precision estimation and systematic error (bias) from the accuracy estimation. The total observed error for the specified analytes were within the allowable error indicated ASVCP guidelines (Table 3). Quality of testing also assessed by sigma metrics. Accordingly all the three anaytes satisfied the recommended requirement (> 3 sigma values). Total cholesterol and total protein showed > 6σ zone (world class quality) while urea showed 4.9σ (Good class quality).The QGI for urea, as it had below 6σ was 0.95 falling in the range of 0.8–1.2 shows both imprecision and inaccuracy (Table 3).

Table 3
The sigma metrics and quality goal index ratio for Total cholesterol, Urea and Total Protein

Analyte	Bias (%)	CV (%)	TE_o (%)	TE_a (%)	Sigma	QGI	Problem
Total Cholesterol	0.54	2.08	4.7%	20%	9.34	0.17	None
Urea	2.68	1.89	6.46%	12%	4.9	0.95	Impression & inaccuracy
Total Protein	0.1	1.63	3.36%	10%	6.1	1.37	None

Discussion

This study was undertaken to determine whether commercial kits are applicable to test analytes in horse serum. There are few studies on the validation of commercial kits and this study is the first in veterinary laboratory environment in Ethiopia. Our study is focused on the recovery and repeatability test results which then followed by calculating sigma values and quality goal index for three analytes namely total cholesterol, Urea and Total Protein in horse serum

According to the findings of the study for the intra-assay and inter-assay precision to be accepted, SD must not exceed 0.25 x TE_a and 0.33 x TE_a respectively for the given analyte [15, 21]. In these regard

intra-assay and inter-assay precision for total cholesterol demonstrated < 5% and < 6.6%, urea < 3% and 3.96%, total protein < 2.5% and < 3.3%. The precision profile representing %CV is within the established acceptance criteria,

The findings of recovery percentages were between expected values and measured values demonstrate that all tests were within the acceptance range of 80%-120% [22, 23]. Besides the error observed was less than the allowable error assigned for the analytes [11]. Quality index ratios for total cholesterol and protein indicates no problem in terms of accuracy and person while in case of urea root for impression and inaccuracy cause should be investigated before it routinely used as the quality of the test in such cases cannot be assured [24, 25].

Conclusion

Validation of the bio analytical methods is an integral part of laboratory management and health care. Commercial clinical chemistry test kits are often validated by the manufacturers. There is a need to verify the validity of the test kits before applying to medical and research purposes particularly when the sample matrix is different. The study demonstrated that the commercial kits used in the study satisfied the acceptable criteria and recommended its use for horse serum. However a full validation a study of the kits for their of fitness for purpose in a number of laboratories and clinical decision limits is recommended.

Limitations

- The study was unable to conduct comparison studies and due to financial constraints
- The study was unable to conduct validation on high and low concentration due to unavailability of materials
- The study was limited to conduct on horse

List Of Abbreviations

ASVCP: American Society of Veterinary Clinical Pathology

OIE: World *Organisation for Animal Health*

QGI: Quality goal index

SPANA: Society for protection of animal's abroad

$TE_{a:}$ Total Allowable Error

$TE_{o:}$ Total Observed Error

Declarations

Consent to publish

The authors give consent for BMC research notes journal to publish the manuscript and it is not *under consideration* for *publication* in another *journal.*

Ethics approval and consent to participate

The study protocol obtained research ethical clearance approved by the institutional animal research ethics committee at the Addis Ababa university college of veterinary medicine and agriculture used in this study (Certificate reference no VM/ERC/09/01/12/2020)

Availability of data and materials

The data used to support this study are available from the corresponding author on request

Competing interests

The authors declare that no competing interests in relation to their work

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Authors' contributions

YC conceived the study and designed it, was responsible for data integrity, analysis and interpretation. YC FR and FT drafted and revised the manuscript. All the authors read and approved the final manuscript.

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