Research article

Analytical method validation of pooled TSH reagent in clinical biochemistry laboratory

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ABSTRACT

Introduction and Aim: In today's world, laboratories are a crucial aspect of health-care services, and the biochemistry section contributes the most testing parameters. To our knowledge, this is the first attempt to verify and validate a pooled TSH reagent kit. The present study is aimed to pool and validate the pooled TSH reagent kit for testing and compare its performance with the intact manufactured supplied reagent kit.

Materials and Methods: Performed in a medium-sized biochemistry laboratory with a daily sample load of 1000-1200. Before disposal, the TSH reagent with the available dead-volume was pooled in a new kit. We gathered three used TSH reagent kits from the same lot series and pooled the dead-volume to generate one functioning reagent. Before assessing the analytical performance of pooled TSH reagent, this was placed onto the instrument, calibrated, and Quality control ran. The CLIA procedure for testing method specification was used to evaluate the reagent's performance, which included accuracy, precision, measurement range, reference range, recovery, interference, and method comparison.

Results: With a high degree of analytical precision, the obtained reference range was within the manufacturer's range (r^2=0.99). Precision was comparable with the manufacturer claim; inter-assay variation (1.90% CV), Intra-assay variation (1.50% CV) and overall (1.33% CV). AMR found to be as true as established by the manufacturer - 0.005-100 µIU/mL. Internal quality control performance; level 2 & level 3 variations of 1.58% and 1.20% respectively.

Conclusion: A pooled TSH reagent was precise, accurate as compared to the manufacturer Reagent kit. This will reduce the number of the reagent procurement by the laboratory with documented high kit utility rate (Kit efficiency Index) ranging between 90-100%.

Keywords: Validation; Verification; Analytical Measuring Range (AMR); Clinical biochemistry laboratory; Pooled Reagent Kit.

INTRODUCTION

The laboratory is a tool for the health care diagnostic approach of evidence-based medical practise for patient care all over the world. It is an ongoing struggle in a health-care system to provide a better diagnostic to assist the treating physician while preserving standard quality norms and credentials. Total Quality Management (TQM) is a continual process that aims to achieve standard quality in all processes involved in patient care in a laboratory. The requirements cover the management's commitment to quality, resource utilisation, quality planning, employee commitment and competence, review of orders, purchase, monitoring of the instruments used in patient care, processes to address the customer complaints, actions taken (corrective/preventive) and continual quality improvement program in laboratory with the drive to achieve better quality at all the aspects.

It is critical to verify the technique from the perspective of patients, laboratory consultants, and regulatory agencies in the health care sector during the initial setup of the test method or product in testing the patient sample to report the results. Laboratory validation is a procedure that ensures the consistency, accuracy, and precision of laboratory test data and outcomes (1). Validation process for the test method, as well as the instrument used must perform the analysis as per the well-established critical specifications as prescribed by the manufacturers at the installation or qualification phases i.e., Installation qualification (IQ), Operational qualification (OQ) and Performance qualification (PQ) protocol (2).

CLIA, CAP, and even ISO 15189 mandate the use of only validated procedures to show that the examination process is acceptable for intended usage. Validation must be as extensive as is required to satisfy the requirements in the field of application, and procedures must be revalidated on a regular basis regarding changing conditions and professional guidance. Laboratory regulations recommend verifying the performance of any new test method at the site of testing before a patient sample is analysed. The performance as specified in the inserts of the reagent includes the precision, accuracy, along with other details include analytical sensitivity, analytical

specificity, linearity range, reference interval, reportable range, interferences, reference range, method comparison, and other applicable characteristics.

It is the duty or responsibility of the laboratory to verify the new method. If the laboratory makes any deviations or the modification in the new method beyond the recommendation by the manufacturer, then the laboratory must validate the performance of the modified method of the validated test method or kit, to derive the new performance characteristics accordingly. In that regard, the current study was conducted in a clinical biochemistry laboratory.

Pooling of the reagent will increase the utility of the reagent kit, as most of the reagent at the end of test assigned in instrument application will be discarded with residual reagent (dead-volume) left over in the kit. If this residual reagent is pooled, we can be in position to prepare a new kit from the pooled empty reagent kits from the instrument. This procedure will reduce the number of reagent kits to be purchased and be helpful in utilising the reagent kit to its 100%. By this it will be an economic support for the medium to large scale laboratory that would be using the special test or the immunoassay reagents at the high turnover rate on day to day. Choosing the reagent for pooling must be decided very carefully and feasible reagents kits with the availability stability onboard. The present study is aimed to pool and validate the pooled TSH reagent kit for testing and compare its performance with the intact manufactured supplied reagent kit.

MATERIALS AND METHODS

Present study was conducted at a medium to large scale biochemistry laboratory with the average sample size of 1000-1200 patient samples per day and conducted for the duration of 10 days; Analysis was done on Roche Cobas 6000 analyser e601 module based on electrochemiluminescence (ECLIA) method (3).

Inclusion criteria

TSH reagent with the onboard stability of 20 days at the time of removal, same lot empty reagent kits with dead volume.

Exclusion criteria

Different lot reagent, expired reagents, reagent kit with onboard stability of less than 10 days at the time of discard.

Procedure

We selected the TSH reagent which fulfilled inclusion criteria to pool. Empty reagent kits with dead-volume of the reagent were collected from time to time in the span of 3 days of the same lot series and stored at 2-8^{o} C in a reagent cooler. Pooled to make one working reagent kit from three used reagent kits with residual dead-volume. The reagents which strictly fulfilled our inclusion criteria were included. Pooled TSH reagent kit was loaded to the Roche instrument Cobas 6000 and assigned position number 06 in e601 section/modulus principle based on electrochemiluminescence. The testing method was calibrated using the traceable calibration for the TSH from the reagent manufacturer Roche, and the Quality control analysed by using the Randox immunoassay control two levels immunoassay level 2 and 3. The TSH levels are reported as µIU/mL.

The regular or routine intact TSH reagent was loaded in a second Cobas 6000 instrument, and the comparison of the method performance of the pooled reagent was done against it. Both the reagents were calibrated at the same time and QC was run in the meantime.

Definitions and measurement

Sample: One or more elements extracted from a system that are designed to offer information about the system, frequently as a foundation for making decisions about the system or its production.

Run: A period within which the trueness and precision of a testing system are assumed to be steady, but which cannot be more than 24 hours or shorter than the manufacturer's specified frequency.

Precision: The degree to which independent test/measurement findings obtained under specified conditions agree. To verify the precision, abnormal samples were processed 2 times a day as replicated for 10 days out of total 70 samples. Intra-assay variations, 20 replicates were run morning and 20 replicates of sample in the evening for the inter-day assay imprecision. Precision can be specified as in (4).

Repeatability: The degree of agreement between the results of repeated measurements of the same measure and taken under the same measurement circumstances.

Reproducibility: The degree of agreement between the results of measurements of the same measure and performed under different measurement settings.

Imprecision: The spread of independent measurements collected under defined conditions. Is calculated by taking the mean, standard deviation (SD), and coefficient of variation (CV) of the data obtained from the analytical runs.

$$CV = (SD \times 100)/mean$$

Intermediate precision conditions

When test or measurement results are achieved using the same procedure, on identical test/measurement items, in the same testing site, but under different operating conditions; NOTES: a) The four elements of operating conditions: time, operator, calibration, and equipment; b) the changed elements in the operating conditions must be noted; this could include precision estimates commonly referred to as ‘between-run’,

'between-day', 'within-day', 'within-device' and 'within-laboratory' (5).

Analytical measurement range (AMR)

The analyte measurement/value range technique may directly measure on the specimen/sample without any dilution or other pre-treatment or preparation that is not part of the standard assay. AMR verification typically consists of three levels: low, mid, and high. Commercial material, proficiency testing samples (PT), or patient samples with established results, standards, or calibrators can all be used.

Analytical method comparison

Here we estimated in duplicates in 40 different patient samples in different ranges of levels-low, mid, high, and very high within the AMR of the reagent across 10 days (6). The comparison between the pooled TSH reagent and the regular reagent TSH levels was done by the linear regression analysis to assess the agreement between the performance of the pooled TSH reagent and TSH reagent (7).

Dilution

Five different patients' samples, whose concentration was more than AMR (>100 µIU/mL TSH) was analysed on the pooled TSH reagent in replicate according to the recommended dilution of 1:10. The bias % between the result of pooled reagent and regular reagent was analysed and should be within the acceptable % bias.

Verification of linearity and recovery

We selected an abnormally high patient sample near to the highest linearity range, and progressively diluted it and measured it to the lower limits & tabulated against the theoretical value. Determination of total error (TE) in TSH measurements using the pooled TSH reagent at each of serial dilutions of sample:

Total error = % Bias + 2*CV%

Pooled sample preparation

The serum samples collected for the testing for the day were pooled in a container (mixed it properly), aliquots were prepared and stored at -20ºC till the TSH was analysed.

Statistics

The study data were represented as the mean, Standard deviation (SD), Coefficient of Variation (CV), linear regression was used to analyse the linearity of measured values between the method/reagents, and student t-test was used to assess the significance of mean difference. Data was entered in the Microsoft Excel & statistical tool SPSS v23 was used where required for analysis.

RESULTS

The pooled reagent was prepared and analysed for the analytical performance using the QC material, EQAS sample and pooled patient serum sample. The study was conducted across 10 days by different technicians to validate the performance of pooled TSH reagent. The conductivity of the RO water during these 10 days was 0.2-0.35µS/cm within the acceptable limit.

Precision

The inter-assay variations were verified in accordance with CLSI protocol (EP15-A2), for 10 days replication of the pooled sample was analysed accumulating the count of 74. Precision gave a CV of 0.90%, comparable with manufacturers' claims. Intra-day assay comprising the 20 replicates in morning and evening, accumulating 40 points provided a CV of 1.57%. The overall CV across the 10 days, precision was found to be 1.33% (Table 1). The daily quality control (QC) performance on Pooled TSH reagent showed a good CV across the 10 days of validating the reagent with 1.58% and 1.20% at the Level 2 and level 3 controls respectively when compared to the regular TSH reagent QC performance with CV of 3.37% and 2.38% at level 2 and 3 respectively (Table 2).

Table 1: The precision of the pooled TSH reagent, across the 10 days of estimation in pooled serum samples

Pooled serum sample	Number (n)	Mean (µIU/mL)	SD	CV%
Intra run	22- morning	7.10	0.12	1.66
	22- evening	7.13	0.11	1.49
Intra Day	44	7.11	0.11	1.57
Inter day	30	7.16	0.06	0.90
Overall	74	7.14	0.09	1.33

Table 2: Daily QC details of the pooled TSH reagent and the routine manufacturer reagent, t-test was used to assess the significance of mean difference

TSH reagent	Quality control	Mean (µIU/mL)	SD	CV%	Range of QC	Sig*
Pooled	Level 2 (n=16)	2.58	0.04	1.58	1.96-2.88	NS
	Level 3 (n=16)	22.38	0.27	1.20	17.7-26.10	NS
Regular	Level 2 (n= 20)	2.60	0.09	3.37	1.96-2.88	NS
	Level 3 (n=20)	22.64	0.54	2.38	17.7-26.10	NS

There was no significant difference between the mean of QC result on the pooled reagent and the regular reagent.
*<.05 statistically significant; NS- not significant.

Accuracy

The two external quality assurance samples were run on the pooled TSH reagent (cycle 5 and 6) of BIORAD EQAS; the deviation from the peer group was compared. The Z-score & Bias% was within the acceptable range of <2.0 (>2.0 considered as outlier) and <7.8 as per the criteria selected to assess the performance (Table 3). Verification of reference range showed the reference range for TSH using the pooled reagent to be 1.1-3.95 µIU/mL which was within the manufacturer's reference range 0.270-4.20 µIU/mL (Table 4).

Table 3: The EQAS performance of the Pooled TSH reagent compared against the peer group and the regular TSH performance

EQAS performance of both the TSH (µIU/mL) reagents cycle 5			
TSH reagent	**Value**	**Bias**	**Z-score**
Pooled	24.05	0.63	**0.19**
Regular	23.7	-0.84	**-0.25**
Peer value	23.9		
Eqas performance is satisfactory			

EQAS performance of both the TSH (µIU/mL) reagents cycle 6			
TSH reagent	**Value**	**Bias**	**Z-score**
Pooled	0.88	3.17	**1.00**
Regular	0.87	1.99	**0.63**
Peer value	0.853		
Eqas performance is satisfactory			

Table 4: The biological variation-desirable criteria of serum TSH testing (8)

aCV %	9.7
aBias %	7.8
TaE	23.7

Linearity

A linear relationship is evaluated across the manufacturer established linearity for the method is 0.005-100 µIU/mL. The verification of the linearity of the pooled reagent at the low concentration and high concentration done with replicates resulting in CV% of 3.71% (low concentration) and 0.60% (high concentration) which are acceptable (Table 5). The linearity was established by the serial dilution of the highest concentration of the TSH sample, and the Total error was estimated respectively. The total error found to be satisfactory and within the recommended range the error was below the allowable total error for the testing method specifications according to biological variation-desirable guidelines (Table 6). The linear equation between the measured and the measured was found to be y = 1.01x + 0.422 with r^2=0.99 (Fig. 1). The total error of pooled TSH reagent at different serial dilution of the known concentration of the TSH sample was found to be lower than the allowable total error (TaE) in the biological variation-desirable specification database (Table 6).

Table 5: The linearity verification of the pooled reagent kit at the low and high concentrate against the TSH reagent

P-TSH (µIU/mL) Value – low conc.		**TSH (µIU/mL) value – low conc.**	**P-TSH (µIU/mL) Value – High conc.**		**TSH (µIU/mL) value – High conc.**
0.032		**0.031**	98.3		**96.98**
0.033			97.63		
0.033			97.63		
0.035			97.18		
0.032			96.67		
Mean	0.033		Mean	97.48	
SD	0.001		SD	0.60	
CV%	3.711		CV%	0.62	

Table 6: Linearity study with serial dilution of patient sample, analysed on pooled TSH reagent

Dilution of sample	**Measured value (µIU/mL)**	**Theoretical value (µIU/mL)**	**Recovery %**	**Bias %**	**CV %**	**TE**	**TaE**
100:0	98.170	98.170	100.0	0.00	1.91	3.82	23.7
90:10	89.650	88.353	101.5	1.47	1.91	5.29	23.7
80:20	79.610	78.536	101.4	1.37	1.91	5.19	23.7
70:30	71.330	68.719	103.8	3.80	1.91	7.62	23.7
50:50	50.990	49.085	103.9	3.88	1.91	7.70	23.7
30:70	30.570	29.451	103.8	3.80	1.91	7.62	23.7
20:80	19.610	19.634	99.9	-0.12	1.91	3.70	23.7
10:90	10.040	9.817	102.3	2.27	1.91	6.09	23.7
0:100	<0.005	<0.005	100.0	0.00	1.91	3.82	23.7

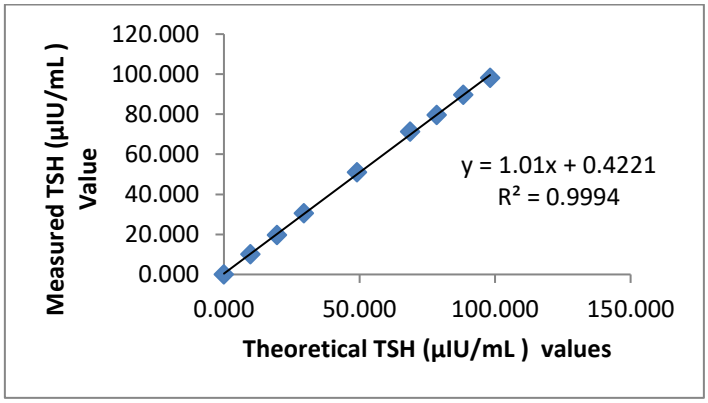

Fig. 1: The linearity of the measured TSH levels against the theoretical TSH level of the same serum sample

Method comparison

The analytical method comparison between the Pooled TSH reagent (y-axis) and the Regular TSH reagent (x-axis) assay in serum samples of the persons under study by linear regression analysis yielded the equation of y=0.998x-0.016, r2 was 0.999 & p<.001. (Fig. 2) Mean bias between the methods was also found to be lower than the acceptable bias 0.002% (acceptable 7.8%) (n=40). (Fig. 3).

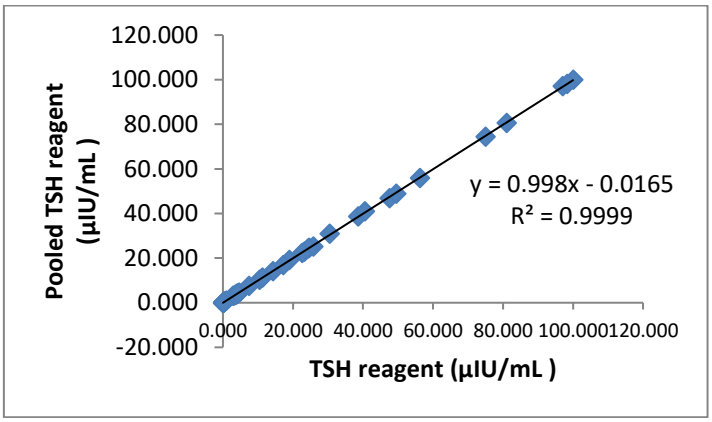

Fig. 2: The analytical performance of the pooled TSH reagent against the regular TSH reagent using the patient sample across the range.

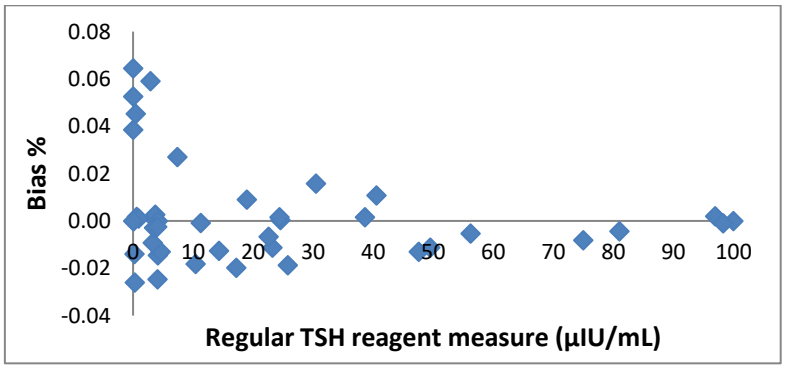

Fig. 3: The analytical mean bias difference between the pooled TSH reagent and regular TSH reagent.

Dilution study

The pooled TSH reagent performance at manufacturer recommended dilutions for patient sample >100 **(**µIU/mL**)** of TSH is 1:10, the resulting bias % was well within the acceptable criteria (Table 7).

Table 7: The concordance of the reagent performance under the recommended dilution for testing

Dilution value p-TSH (µIU/mL**)**	**Dilution value TSH (**µIU/mL**)**	**Bias %**	**Allowable Bias %**
387.4	381.8	0.015	7.8
125.6	124.9	0.006	7.8
362.4	354.8	0.021	7.8
206	205	0.005	7.8
103.1	102.6	0.005	7.8

The Bias % of the testing method with the recommended dilution (1:10) is acceptable as it is lower than the allowable bias % according to the Biological Variation-Desirable database.

Pooled TSH kit utility summary: at the end of the successful validation of pooled reagent for calibration, quality control, EQAS sample, patient sample, pooled serum sample, linearity study, method comparison. The kit reutilisation by pooling of the 3-reagent kits found to analyse 182 tests against the expected 200 test per reagent kit: with the 91% reutilisation rate (Table 8).

Table 8: The summary of TSH kit utility from pooled reagent at the end of validation

Purpose	**Reagent shots utilised**
Calibration	4
QC	32
pooled sample	74
patient sample verification	43
dilution of sample	10
verification of linearity	8
linearity establishment	9
Eqas sample analysis	2
Total No of test performed	182
Expected no of test per TSH kit	200
Kit utility (%)	91 %

Interference: we have not performed the interference study in our study, but the manufacturer of literature describes the assay is unaffected by haemolysis (Hb < 1 g/dL), icterus (bilirubin < 41 mg/dL), biotin < 25 ng/mL, lipemia (Intralipid < 1500 mg/dL), IgM< 0.5 g/dL and IgG < 2 g/dL. Considering the same makeup of the reagent, the above interference specifications might hold good for the pooled TSH reagent as well.

DISCUSSION

Validation of analytical test methods is done when the new method is identified or modified in an established testing method. In this work we have validated the modification in technique of pooling reagent for analytical precision, accuracy, variations in inter and intra-assay, limits of detection, linearity, dilutions, and reportable range. We have used the pooling of TSH reagents for above validation. Our reference range was found to be within the manufacturer's reference range. The comparison of analytical accuracy between the pooled and regular reagent gave, $y = 1.01x + 0.422$ with $r^2 = 0.99$, establishing a linear correlation (very high degree) of and validation of pooled TSH reagent. The precision variations from the 74 replicates (overall variations) were 1.3% (CV%), in concordance with manufacturer's claims. Similarly, the Intra assay variations were also within acceptable limits (CV=1.57%).

Analytical measuring range was verified, different analytical range patient samples were comparable with the routine TSH reagent concordance with the manufacturer's claims. CV% of 3.7% and 0.62% for the lowest concentration and the highest concentration respectively. Study showed a good linearity at different dilutions; also, the recovery test results were within acceptable limits of 90-110%, with $y = 1.01x + 0.422$ with $r^2 = 0.999$ between the theoretical (expected) and measured (observed) values of TSH.

It is critical in today's era of technology innovations and biochemical laboratories to ensure the quality of the entire testing method validation process. The pooled reagent showed in par performance with the manufacturer supplied reagent kit, hence cutting down the number of reagent kits being purchased which will not only reduce the budget for the laboratory but also can levy the percentage charges on the patient test samples. This will reduce the cost per test (CPT), in this case we can get one new pooled reagent kit using the dead-volume from the 3 discarded TSH reagent kits. The kit utility percentage in our study is between 90-100%, which is an impressive number, with 182 tests performed against expected 200 tests from the reagent kit. Because of this effort, we were able to execute systematic validation of the pooled TSH reagent kit, resulting in much improved analytical performance and more reliable and accurate reporting of patient test results. To the best of our knowledge, no such method or the process of pooling a reagent has been reported in the literature.

Method validation is a critical step towards the quality of the entire process in today's sophisticated biochemical testing facilities. A laboratory consultant verifies or identifies any flaws in the process by reviewing the analytical data. As a result, the quality of evidence and evidence-based laboratory practices must be confirmed (9).

Recommendations

The following points are mandated to be considered during pooling of reagents for the analysis of patients' samples.

a. Reagent stability (onboard and offboard).
b. Reagent integrity.
c. Reagent dead-volume / after use volume.
d. Calibration of new reagent.
e. Lot sequence number of reagents.

f. Number of days, the discarded reagent stored at 2-8º C for pooling.
g. Ease of pooling.
h. Turnover of reagents at lab (Highly preferable).
i. Pooled reagents must pass the calibration, QC (EQAS if available) and patient sample verification before applying for patient sample testing.

CONCLUSION

We conclude that a pooled TSH reagent is accurate and precise in concordance to the manufacturer reagent kit. This method can be used for the reagents having the high turnover at the laboratory setting such that reagents have enough stability onboard to be considered for the pooling. This will reduce the number of the reagent procurement by the laboratory with the high kit utility rate (Kit efficiency Index) ranging between 90-100%. This can be subjected for all the reagents which can be easily transferable. The number of utilised reagent kits needed to be pooled for making one functional reagent kit varies with the different testing reagent kits, manufacturers and should be standardised for all the testing reagents before analysing patients' samples.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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