Analytical Performance evaluation of the New VITROS TSH3 assay on VITROS XT 7600 Integrated System

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Introduction

Thyroid stimulating hormone (TSH) has been used extensively as front-line test for the initial screening of patients to distinguish euthyroid status from both hyperthyroidism and hypothyroidism. Endocrinologists use the combination of TSH and thyroid hormones – Thyroxine (T4) and Triiodothyronine (T3) either free form or total, both free and bound forms together as thyroid function tests. While doing thyroid function tests, changes in the TSH level can serve as an 'early indicator' before the actual changes in the level of thyroid hormones (T3 and T4) in the circulation (Sheehan MT, 2016). The high level of TSH indicates hypothyroidism where the thyroid gland is unable to make sufficient thyroid hormones and low level of TSH indicates hyperthyroidism where the thyroid gland is making high level of thyroid hormones with some exceptions (Guerri G, 2019). There are multiple assays available for the measurement of TSH but there is not any harmonization between different methods. Systematic difference between various assays may produce misleading interpretation when samples of the same patients are measured using different assays based on different methodology (Clerico A, 2017). The IFCC Committee for Standardization of Thyroid Function Tests developed a global harmonization approach for TSH measurements using standards or calibrators which are traceable to APTM (All Procedure Trimmed Mean) – a panel of native materials with values assigned by the APTM as the surrogate Reference Measurement Procedure (Thienpont LM, 2017). Ortho Clinical Diagnostics introduced New VITROS TSH3 assay which is traceable to APTM Reference serum panel. The objective of this study is the evaluation of new VITROS TSH3 assay for its usage in our Thyroid Function tests panel.

Materials and Method

This analytical evaluation study was conducted at M/s Toprani Advanced Lab Systems, a NABL Accredited Pathology Laboratory in Vadodara, Gujarat, India. VITROS TSH3 reagent pack was calibrated in three VITROS XT 7600 Integrated system as per the manufacturer's Instruction for use manual and the calibration verification was done by processing three levels of Bio-Rad Immunoassay control and the obtained results were compared with the peer group mean value.

The accuracy and precision verification of VITROS TSH3 assay was carried out following the CLSI EP 15 A3 guidelines using two levels of VITROS Total Thyroid Immunoassay control samples, both Level 1 and Level 3 controls. Each sample was processed in five replicates in a single run, and 5 different runs in three days. The co-efficient of variation (CV%) was calculated and compared with the upper verification limit (UVL) of the manufacturer's performance characteristics of the assay in terms of repeatability and reproducibility as specified in the VITROS TSH3 Instruction for use manual.

The Analytical measurement range (AMR) of the VITROS TSH3 assay was verified by following the CLSI EP 06 guidelines. A linearity panel of 11 samples were prepared by proportional mixing of both high and low value samples in different proportions viz., 1:9, 2:8; 3:7;4:6; 5:5; 6:4; 7:3; 8:2; 9:1 and having low value sample as level 1 and high value sample as level 11. All the 11 samples were processed in duplicate, and the obtained value was compared with the mathematically expected value. The obtained results were plotted graphically with the expected value in the x-axis and the obtained value in the y-axis. The assessment criteria for the AMR verification was the visual examination of the plots for any potential outlier at any of the concentrations and the correlation coefficient (r).

The performance of VITROS TSH3 assay in clinical samples were evaluated by processing patient serum samples in both, the existing VITROS TSH assay and the new VITROS TSH3 assay simultaneously. A total of about 150 patient serum samples were collected randomly covering the analytical measurement range of the VITROS TSH assay, ranging from 0.1 to >100 µIU/mL based on the value obtained in the VITROS TSH assay. All the 150 samples were processed in the VITROS XT 7600 system in both VITROS TSH and VITROS TSH3 assay in the same system within an hour. The Passing - Bablok regression analysis was carried out to verify the comparability between both the assays. Further, to verify any statistically significant variation observed between the values obtained with both VITROS TSH and VITROS TSH3, the paired t-test was done.

Results

VITROS TSH3 assay was calibrated in three VITROS XT 7600 Integrated systems and verified using three level Bio-Rad Immunoassay controls. The obtained results were compared with the peer group mean value and found to be satisfactory (Table 1).

Table 1: Calibration verification of VITROS TSH3 assay.

Control Level	VITROS XT7600 - 1	VITROS XT76 00 - 2	VITROS XT7600 - 3	Peer Group Mean	Peer Group SD	Peer Group Range
Level 1	0.35	0.37	0.38	0.37	0.02	0.33 - 0.41
Level 2	4.84	4.70	4.78	4.74	0.16	4.42 - 5.06
Level 3	33.85	34.39	34.19	32.88	1.23	30.42 - 35.34

The analytical performance of VITROS TSH3 assay for accuracy and precision was verified using CLSI EP15A3 Guidelines. The verification study was performed using two concentrations of VITROS Total Thyroid Quality control samples (Level 1 and Level 3) in all the 3 VITROS analyzers and 25 replicates of QC samples per analyzer in 3 days. The Trueness or accuracy verification was done by calculating the Bias% between the obtained mean value of 25 replicates and the target value given by the manufacturer for the control samples. VITROS TSH3 assay showed results which are comparable with the target value

Table 2: VITROS TSH3 – Accuracy verification.

VITROS XT 7600	Control Level	Target value (uIU/mL)	Range (uIU/mL)	Obtained (uIU/mL)	Bias %	Allowable Bias %
System 1	Level 1	0.078	0.062 – 0.094	0.073	6.41	7.80%
System 2				0.076	2.56	
System 3				0.082	5.13	
System 1	Level 3	19.68	16.33 – 23.03	19.1	2.95	
System 2				19.45	1.17	
System 3				19.81	0.66	

Imprecision estimates in the form of within-run or intra-assay precision (repeatability) and within-lab or inter-assay precision (reproducibility) were calculated in terms of CV% and compared. The precision study showed an acceptable inter and intra-assay precision when compared with the manufacturer's claim and the upper verification limit (UVL) (Table 3).

Table 3: VITROS TSH3 Inter and Intra assay Precision verification.

VITROS XT 7600	Control Level	Conc. (uIU/mL)	N	Repeatability (Intra assay Precision)			Reproducibility (Inter-assay Precision)		
				Obtained	Claim	UVL	Obtained	Claim	UVL
System 1	Level 1	0.07	25	1.30%	2.10%	2.80%	2.10%	4.60%	7.10%
System 2		0.08		1.10%			1.20%		
System 3		0.08		1.50%			2.20%		
System 1	Level 3	19.1	25	1.70%	2.70%	3.50%	2.30%	6.50%	10.40%
System 2		19.45		1.50%			2%		
System 3		19.81		3.00%			3.40%		

Analytical measurement range or linearity verification was carried out following the EP 06 guidelines. A linearity panel of 11 samples with expected value ranging from low to high (0.028- 147.32 µIU/ml) were processed using VITROS TSH3 assay on VITROS XT 7600. Each sample was run in duplicate and an average value was generated using the two values obtained. A regression graph was generated between the expected value and the obtained value for all the 11 samples. There was a significant correlation between the expected and the obtained TSH values as indicated by the slope of the graph (R2=0.9947), which verifies the linearity of the assay (acceptance criteria: CI 95%, linear regression r=0.950-1.00) (Fig 1).

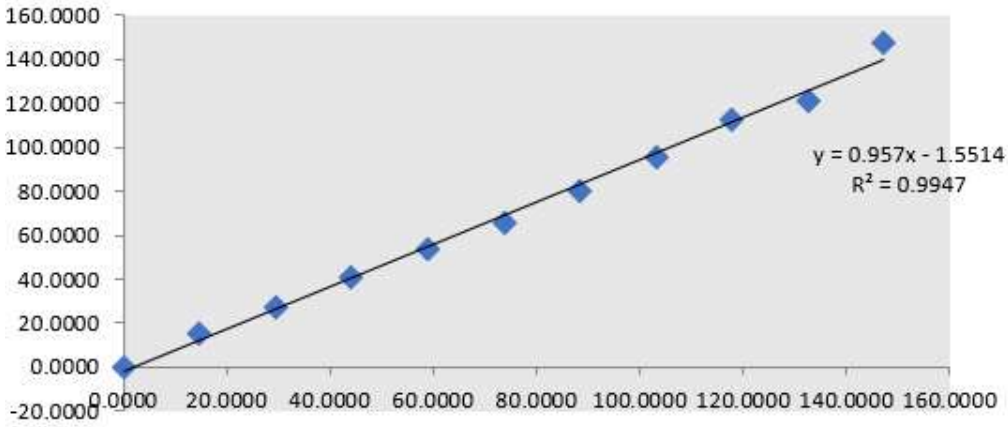

Fig 1: Linearity verification of VITROS TSH3 assay.

To evaluate the usefulness of the VITROS TSH3 with enhanced linearity up to 150 µIU/mL, a total of about 150 patient samples were analyzed using VITROS TSH3 along with VITROS TSH assay. For the sample comparison study, patient samples were selected from hyperthyroid (10 subjects), euthyroid (67 subjects) and hypothyroid (73 subjects) patients based on the values obtained with VITROS TSH assay. The mean serum TSH levels of hyperthyroid patients (10 subjects) was determined as 0.07± 0.09 and 0.09± 0.11 µIU/ml using the VITROS TSH and VITROS TSH3 assays, respectively. The mean serum TSH level of euthyroid patients (67 subjects) was determined as 2.53 ± 1.26 and 2.54 ± 1.29 µIU/ml using the VITROS TSH and VITROS TSH3 assays, respectively.

The mean serum TSH level of hypothyroid patients (64 subjects) was determined as 20.01 ± 21.86 and 22.60 ± 28.91 µIU/ml using the VITROS TSH and VITROS TSH3 assays, respectively. Overall, in all the 150 samples, a strong positive correlation was found between the results of the two assays (r=0.983) (Fig 2). As per the paired t-test, there was no statistically significant difference observed between the values ($p < 0.05$). The clinical interpretation of most of the subjects was similar in both the methods used, with 12 subjects out of 154 samples showing borderline hypothyroid status when evaluated by the VITROS TSH3 assay who were otherwise euthyroid when the VITROS TSH assay was used. This is mainly because of the variation in the reference range recommended in the Instruction for use manual of both the VITROS TSH3 (0.40 – 4.049 uIU/mL) and VITROS TSH (0.465 – 4.68 uIU/mL) assays. The regression analysis shows that there is a positive bias with the TSH3 assay when compared with the TSH assay (Y intercept – +1.116). The application of regression statistics at the clinical decision point of 5 µIU/mL shows that the percentage of difference at the medical decision point is 24.8 % which is less than the maximum allowable TeA % as per BV guidelines (38.2 %). Hence, the comparison correlates well both clinically and statistically.

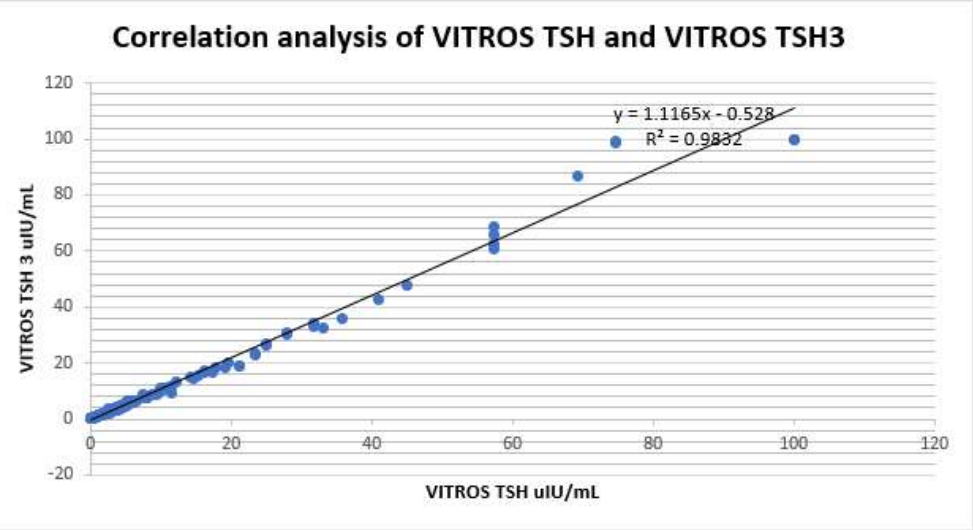

Fig 2: Correlation of VITROS TSH and VITROS TSH3 assays

One of the advantages of the VITROS TSH3 when compared to the VITROS TSH assay is that there is no biotin interference in the VITROS TSH3 assay. In the assay architecture, since the biotin-BSA coated wells are saturated with streptavidin-labelled mouse monoclonal anti-β subunit of TSH, there is no binding of endogenous biotin and, hence, no interference in the assay, whereas the VITROS TSH assay has the limitation of biotin interference at the concentration of 12.5 ng/mL (Ali M, 2017). Recently, we received a sample having a TSH value of > 100 µIU/mL in other commercially available assays showing a falsely low TSH value of 9.2 µIU/mL in the VITROS TSH assay on a neat serum sample, but using a dilution of 1:5 obtained a value comparable to the value obtained with commercially available assays. When the same stored sample was retested in the VITROS TSH3 assay along with the VITROS TSH assay, the sample showed a value of 124.2 µIU/mL in the VITROS TSH 3 assay and 4.64 µIU/mL in the VITROS TSH assay, indicating there is no interference in VITROS TSH3 assay when compared to VITROS TSH assay.

Discussion

TSH is an important assay for the evaluation of thyroid function (Sheehan MT, 2016) and has been recommended by recent guidelines as the first-line test for evaluation of thyroid hormone related disorders (Dittadi R, 2017), (Garber JR, 2012). This has led to the development of improved TSH assays with enhanced analytical sensitivity and reproducibility (Clerico A T. T., 2018). To harmonize results across different assays, the IFCC Committee for Standardization of Thyroid Function Tests has introduced APTM Reference serum panel (Thienpont LM, 2017).

The new VITROS TSH3 is a third-generation assay with a functional sensitivity of 0.01 µIU/mL, inter-assay CV of 20% and is traceable to the APTM reference serum panel. In this study, we have evaluated the usage of the new VITROS TSH3 assay in our thyroid function tests panel. The analytical performance of VITROS TSH3 assay was verified using CLSI Guidelines. Precision verification and accuracy verification showed an acceptable performance of the VITROS TSH3 immunoassay for both inter and intra-assay precision with the CV% below 3.5% and comparable to the manufacturer's claim. The VITROS TSH3 Assay passed the linearity verification acceptance limit and demonstrated excellent linearity.

High affinity of biotin-streptavidin interaction has been explored in in-vitro immunoassays. Despite the achievement of improved analytical sensitivity of the immunoassays using biotin-streptavidin complex, these assays are prone to interferences with endogenous biotin molecules, as biotin is commonly used for preventing and treating biotin deficiency associated with pregnancy, malnutrition, rapid weight loss, hair loss, brittle nails, skin rash in infants, diabetes, etc. Such interferences can affect clinical decisions and may lead to misdiagnosis and/or inappropriate management of the patients. New VITROS TSH3 Assay utilizes the biotin-streptavidin complex to enhance the sensitivity but without any endogenous biotin interference with the improved assay architecture and, thus, enhances the accuracy of the assay results which helps in enhancing clinical diagnosis of thyroid disorders.

Comparison of New VITROS TSH3 Assay with the currently available VITROS TSH Assay (a third generation Assay) was done to evaluate the clinical usefulness of the more sensitive assay. TSH levels in a broad range of donors (hyperthyroid, euthyroid, and hypothyroid) were compared using the two assays. Regression analysis showed a good correlation between the two assays. There was a good correlation with the clinical diagnosis of thyroid disorders in the subjects for both methods. When compared with the VITROS TSH assay, the VITROS TSH3 assay has an enhanced analytical measurement range (100 µIU/mL vs. 150 µIU/mL) which helps in getting more reliable results for all the hypothyroid patients. Thyroid hormones are essential for growth and development of the fetus during pregnancy and during infancy to childhood as well as regulating metabolic hemostasis. The VITROS TSH3 assay with the established trimester-specific pregnancy reference intervals as well as age-specific reference intervals from infancy to adulthood helps in the correct interpretation of individual TSH levels which aids in the diagnosis and management of thyroid diseases.

The adult reference interval for the VITROS TSH3 assay (0.4 – 4.049 µIU/mL) provided in the VITROS TSH3 Instruction for use manual is consistent with the range recommended by the American Thyroid Association (ATA) guidelines and the statement issued by the British Thyroid Association Executive committee (Jonklass, et al., 2014; Okosieme, et al., 2016. In our study, with the revised reference range, about 12 subjects showed borderline hypothyroid status when evaluated by VITROS TSH3 assay who were otherwise euthyroid when the VITROS TSH was used with the reference range of 0.465 – 4.68 µIU/mL). Further follow-up of those 12 patients will be required to assess their thyroid status, based on the mildly elevated TSH values in relation to the revised reference range. The prevalence of subclinical hypothyroidism is up to 11.3% in India (Deshmukh et al., 2013).

Conclusion

In our study, the VITROS TSH3 assay showed excellent analytical and clinical performance in the comparative study with the current VITROS TSH assay. In addition, VITROS TSH3 has the added advantage of enhanced analytical measurement range, established reference range for trimester-specific pregnancy terms of gestation as well as for the paediatric population from infancy to adulthood, with the calibration stability up to 40 days. The reduced turnaround time from 37 min to 24 min is an added advantage. Furthermore, there is no interference from endogenous biotin in the VITROS TSH3 assay. Our study demonstrated the superior performance of VITROS TSH3 assay and it is incorporated in our Thyroid Function Test panel of assays.

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