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Revalidation of analytical performance and precision of pituitary hormones, TSH, FSH and LH by electro-chemiluminescence immunoassay technology (iECL) and 3rd Gen analytical methods.

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1. Abstract:

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Background: In last two decades, several professional organizations and clinical laboratories carried out validation, revalidation, precision analysis and intra-assay comparison studies for hormonal assays in addition to routine chemistry parameters. The rationale was to manage, control and ensure precision, quality and prompt turnaround time (TAT) for laboratory services regarding highly significant endocrine chemistry reporting. *Aim and Objectives*: The present study was embarked on to re-validation our existing IFCC guided iECL analytical methods which is now governed by 3rd Gen principles. Three pituitary hormones were chosen, viz TSH, FSH and LH and its revalidation was performed by precision comparison and analytical repetition on three college of American pathology (CAP) accredited equipments Cobas e601, Cobas e411 and Elecsys 2010 (Roche Diagnostics). *Methods:* The study was carried out at Department of Biochemistry Lab services and Chemical Pathology, Liaquat National Hospital and Medical College. Retrospective data was collected for the period Jan 2014 to Dec 2015 from departmental data archives (LIS-lab information system). Parametric components, TSH, FSH and LH, n = 60 each, divided into n = 30 Preci-control normal PNU normal range, n = 30 and Preci-control Pathological PPU pathology range. Process of validation and revalidation were completed with evaluating and assessing the precision analyzed Preci-control data from various instruments (modular or standalone, conventional or advanced) and plotting against each other with regression analyses R^2 (see statistical analyses) as per established guidelines. *Results and Conclusion*: Results showed considerable revalidation assessed through regression analysis R^2, ranging from 0.898 to 0.998. Revalidation ensured 89.8% to 99.8% accuracy and exactness of results, whether analyzed on one type of instrument or another.

Key Words: International federation of clinical chemists (IFCC), electro-chemiluminescence immunoassays (iECL), 3rd Gen (generation), College of American pathology (CAP).

2. Introduction:

Since the emergence and development of newer, faster, operator-friendly and economically feasible equipments for clinical laboratories in last three decades, pressure to manage, control and ensure precision, quality and prompt turnaround time (TAT) is getting stronger [1-8]. It has been reported that during late 1990s, European committee for clinical laboratory sciences (ECCLS) developed basic concept of analytical evaluations, which is based on three guiding principles, evaluation of prototypes, multicenter evaluation and validation [6-10].

In this regard, several professional organizations and clinical laboratories carried out validation, precision analysis and intra-assay comparison studies for hormonal assays in addition to routine chemistry parameters [5, 6, 11-15]. The hormones that were more precisely selected includes hormones of pancreatic, parathyroid, thyroid and pituitary origin due to its significant clinical implications and variable reference ranges [5, 6, 12, 15, 16].

The present study reports re-validation of existing international federation of clinical chemists (IFCC) guided electro-chemiluminescence immunoassays (iECL) and 3rd Gen (generation) analytical methods of three pituitary hormones, TSH, FSH and LH by precision comparison and analytical repetition on three college of American pathology (CAP) accredited equipments Cobas e601, Cobas e411 and Elecsys 2010 (Roche Diagnostics).

3. Materials and Methods:

Study Period and Protocols: 1-*Setting*: The study was carried out at Department of Biochemistry Lab services and Chemical Pathology, Liaquat National Hospital and Medical College. 2-*Study type*-Retrospective data was collected for the period Jan 2014 to Dec 2015 from departmental data archives (LIS-lab information system). 3-*Sample/data volume and parameters*: Parametric components (n = 60 each, divided into n = 30 PNU normal range, n = 30 PPU pathology range) of three pituitary hormones, thyroid stimulating hormone (TSH), Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were used to re-validate our existing standardized IFCC regulated analytical techniques on several automated immunological analyzers namely Cobas 6000 e601, Cobas e411 and Elecsys 2010 with Electro-Chemiluminescence immunoassay (ECLi) techniques. Process of validation and revalidation were completed with evaluating and assessing the precision analyzed Preci-control data from various instruments (modular or standalone, conventional or advanced) and plotting against each other with regression analyses R^2 (see statistical analyses) as per guidelines described earlier [7,9].

Analytical methods:

Analytical methods that were used to process and examine parametric components of hepatic and thyroid function tests were also CAP, IFCC, AACC and CLSI accredited and regularized inclusive of instruments. Precision data were retrieved for all

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individual parameters determination that was optimized through 30 consecutive run on respective instruments. All parameters were auto-analyzed by standard methods as established earlier for TSH [17, 18], FSH [19] and LH [20]. Normal reference ranges of TSH was 0.27-4.2 µIU/ml; FSH 1.5-12.4 mIU/ml and LH 1.7-8.6 mIU/ml, respectively.

Statistical and regression analysis: For re-validation, precision analytical methods were used as confirmatory tools, where data resulting either from patient's samples or Preci-controls analyzed on various instruments were plotted as Regression analysis R^2 [9]. R^2 that showed values greater than 0.90 or 90% was considered as satisfactory.

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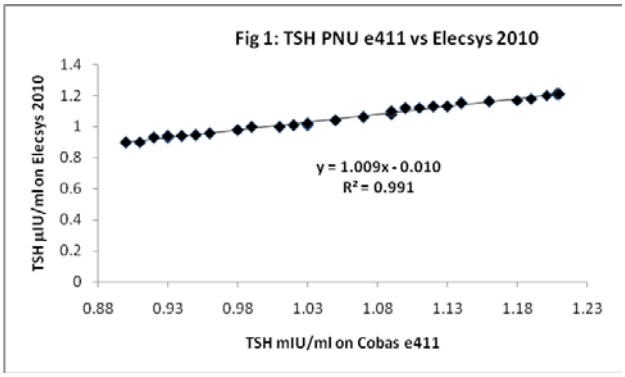
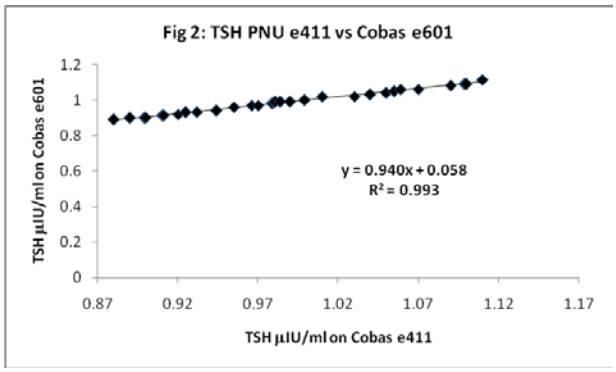
4. Results:

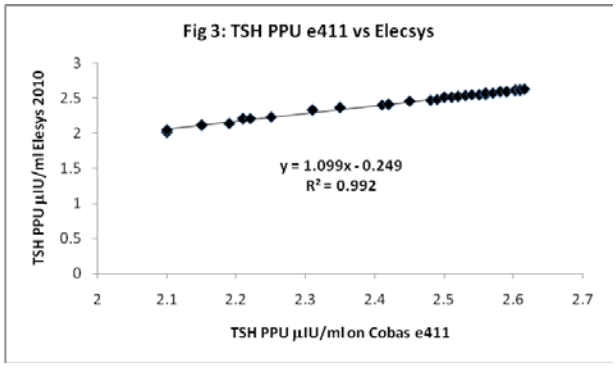
Results are summarized in Fig 1 to 12. Revalidation was performed by multiple analyses of PNU and PPU controls of three pituitary hormones, TSH, FSH and LH on iECL immunoassay technology instrument of Cobas e411, Cobas e601 and Elecsys 2010. Each precision analyses comprised of 30 consecutive runs of either PNU or PPU of each hormone on individual instruments such that comparative groups of 2 pairs [Cobas e411 vs Elecsys 2010 and Cobas e411 vs Cobas e601] were obtain. Comparative precision analysis as per revalidation outcome exhibited R^2 efficiency and accuracy of 0.986 to 0.998 linear regressions. Revalidation of TSH by PNU analyses showed precision and linear regression of 0.991 on e411 vs Elecsys and R^2 0.993 on e411 vs e601 (Fig 1 and 2). However further validation through PPU exhibited R^2 of 0.992 and 0.994 respectively for same group of comparative instruments (Fig 3, 4). Interestingly FSH revalidation outcome was similar in both groups of comparative instruments combo, which was 0.998 thus exhibiting precision validation of upto 99.8% (Fig 5-8). Revalidation of LH showed variable R^2 from PNU on each of two comparative instrument groups, 0.991 and 0.989 (Fig 9, 10) whereas R^2 of 0.986 and 0.993 respectively for PPU precisions (Fig 11, 12).

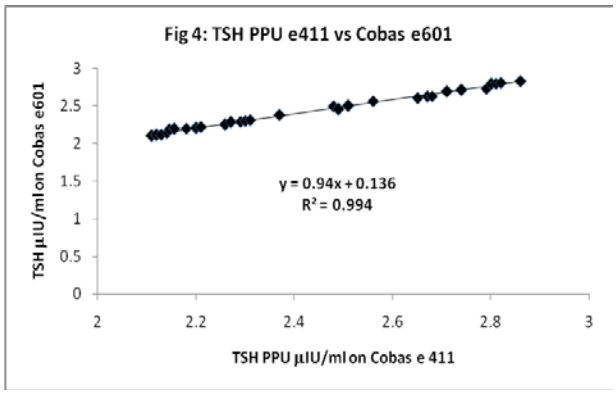


5. Discussion:

In practical terms, rationale to proceed for method, instrument, analytical principle validation or comparative precision analysis was not clearly defined or discussed in scientific research. Moreover, both validation and verification methods, either for evaluation of analytes, instruments, reagents or any method are advocated to strengthen existing protocols or if assessing the new ones [9]. Subsequently, as early as 1990s, Federal drug agency of USA commences the initiatives and programs for bio-analytical validation of methods and analytes [9, 21, 22]. Furthermore, referring to its counterpart in US, the European Union also started working on validation and verification protocols [23] as early as 2011 known as "European Medicine Agency Guidelines on validation of bioanalytes methods". Accuracy, in addition to validation and verification is one of the significant parameter to prioritize precision analysis and comparative evaluation [24]. As per clinical requirements, methods, analytes, instruments must be evenly related and consolidated for accurate outcome according to the values defined for a particular analyte in healthy population [25, 26] or pathologically diseased [27].







In this regard, our current study reported revalidation of precision analysis and analytical performance of three pituitary

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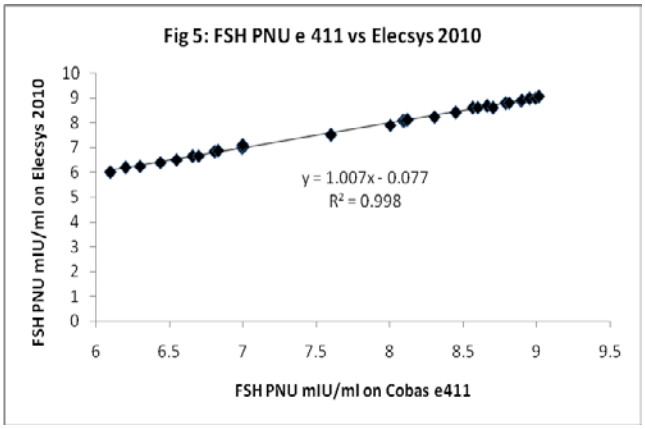
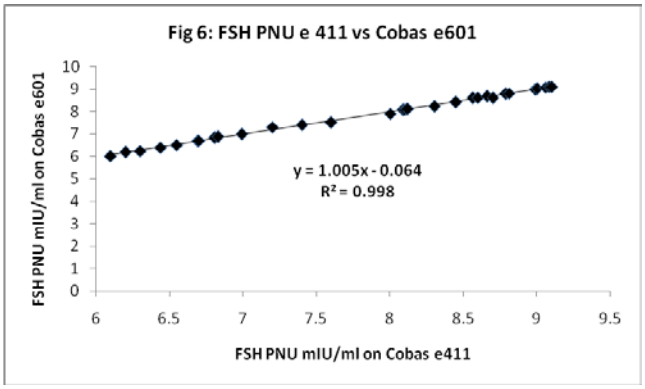
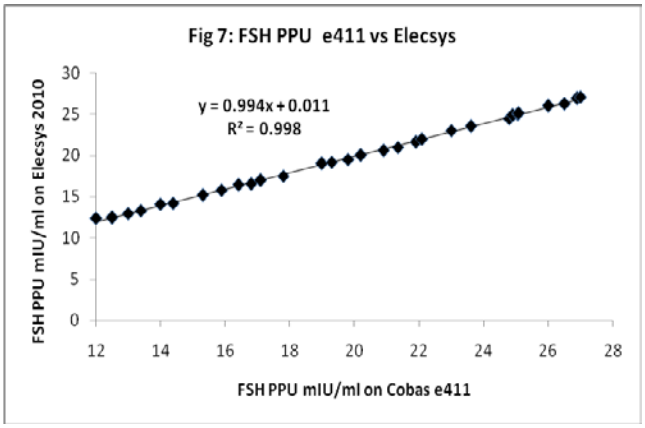
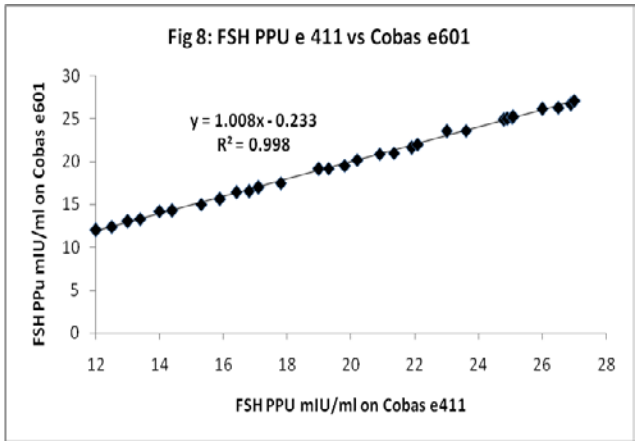
hormones, viz TSH, FSH and LH, on three of our College of American (CAP) certified instruments by precision and accuracy comparison. Our data showed concomitant precisions and subsequent accurate revalidation of TSH, FSH and LH, ranging form R^2 0.991 to 0.994, 0.998 and 0.898 to 0.993, respectively. Previous studies on TSH reported good sensitivity, specificity and accuracy based on ROC as per patients from Hyperthyroidism and hypothyroidism [6]. Similarly inter-laboratory validation of FSH showed measurement variations when long-term storage of samples was compared for analytical recovery [5]. Moreover EIA procedure for LH analysis exhibited 50% relative binding sensitivity at minimal volume [12].

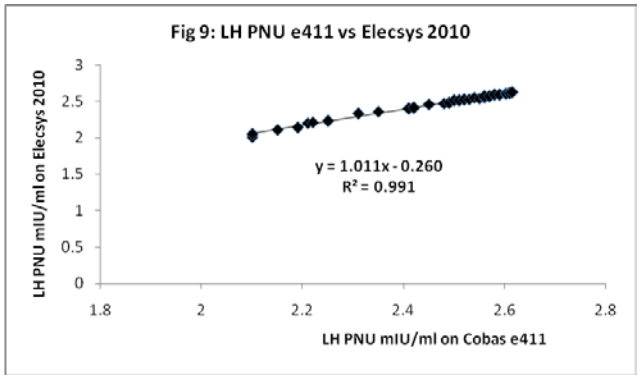
It has been reported that by an average clinical laboratories analyze 20 measurable components from a single person [7]. If theoretically assessing this average measurement per person, thus the total tally would be 200 million tests in developing countries population per year. This huge task is only possible because of availability of high-tech advanced level Gen 3, validated and precision-determined instruments 24/7 [7]. Due to this achievement, in last two decades, labs reduced its repeatability, variations, bias and errors [11].

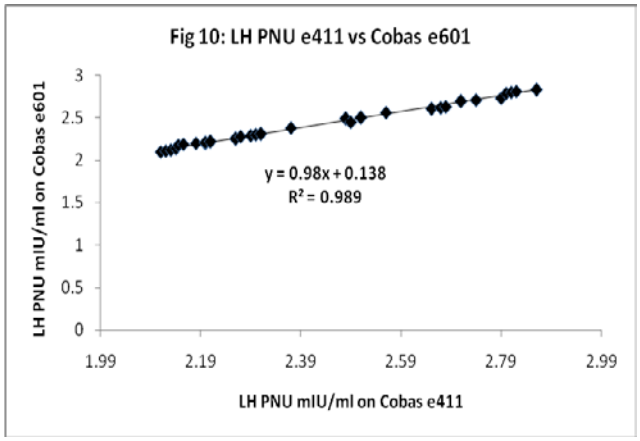












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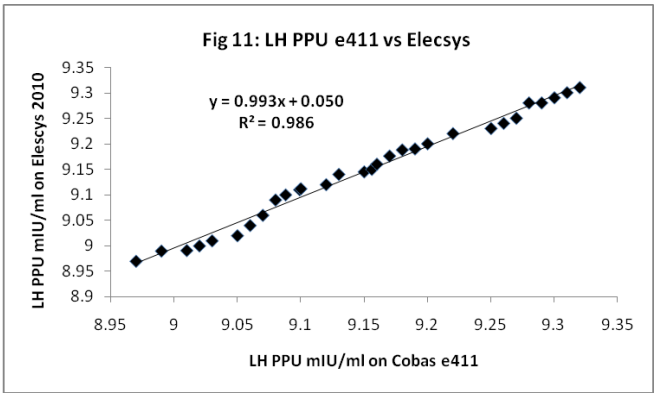
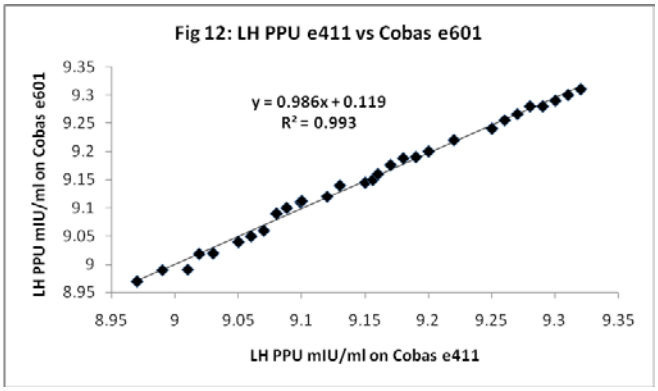
1. Conclusion:

In present study, we reported re-validation of three pituitary hormones, viz TSH, FSH and LH through comparative precision analysis on three iECL Gen 3 instruments and methods. Results showed considerable revalidation assessed through regression analysis R^2, ranging from 0.898 to 0.998. Revalidation ensured 89.8% to 99.8% accuracy and exactness of results, whether analyzed on one type of instrument or another.





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