Accuracy Reference Standards for Quantitative Assays of Thyroid Hormones: Impacts on Clinical Reference Ranges

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1. Introduction

Disorders of thyroid metabolism affect millions of patients worldwide. Clinical diagnosis and treatment requires testing and monitoring of thyroid hormone levels. Reference ranges for thyroid hormones vary among patient subgroups and disease states. Traditionally (T3), T4, and TSH were the most commonly measured thyroid hormones. Reverse triiodothyronine (rT3) has also been recognized as an important thyroid hormone. Vitamin B12, folic acid, and iron deficiency anemia can alter thyroid function, presenting challenges for determination of thyroid hormones in clinical practice [1].

2. Availability of Reference Materials for Thyroid Hormones

Triiodothyronine (T3) and thyroxine (T4) are the most commonly tested thyroid hormones. Accurate Reference Standards for Accurate Quantitation of Thyroid Hormones: Impact on Clinical Reference Ranges

3. Synthesis and Purification of T3 and T3

• Commercially available T3 was purchased from Sigma Aldrich and tested at Cerilliant. Material was found to have low purity and a significant mass balance purity factor.
• Cerilliant synthesized T3 by selective de-iodination of T2, by hydrogenation, followed by terminal ring iodination with iodine and potassium iodide, and purification by reverse phase column chromatography. Challenges to purification included separation of related impurities T2 and T4, and removal of inorganic content.

4. Collaborative Study between LabCorp and Cerilliant

LabCorp evaluated the Cerilliant T3 Certified Spiking Solution® by LC/MS/MS at two sites, Burlington, NC (CET), and Esoterix, Inc. (ESO). Initial results indicated the spiking solution was spiked at 30 % to high relative to a calibrator prepared in-house from powder T3 obtained from Sigma-Aldrich.

5. Certification of Thyroid Hormone Neat Materials and Spiking Solutions

- T3 synthesized and T3 purified by Cerilliant were certified for use as CRM's by full characterization of the chromatographic purity, identity, residual impurities, and residual and impurities.
- Mass balance solutions were prepared mass balance purity values.
- Neat solutions were purified and characterized for total impurities (i.e., the mass fraction of the analysis of interest), certified with an internal standard, based on molar response of proteins in the NMR spectrum.

6. Synthesis and Purification of T3 and T3

- The larger discrepancy between purity factor and CRM purity assay for T3 offers additional challenges for QMRCs, particularly with T3. While T3 is distributed as a quantitative RM, the large variation in purity factor could be an issue for laboratories using T3 for clinical studies.

7. Collaborative Study between LabCorp and Cerilliant

LabCorp tested the Cerilliant T3 Certified Spiking Solution® by LC/MS/MS at two LabCorp sites.

8. Clinical Reference Ranges: Transformation of Reference Intervals

- Changes in clinical reference ranges are needed as a result of improvements in laboratory medicine, compensation for co-morbidity, and the increasing use of low reference range materials.
- A study over multiple days and multiple batches was conducted to obtain a transformation equation for LabCorp current reference intervals using the EP User Table B.
- The resulting slope and intercept differences (p<0.01) was used to transform the existing reference intervals (based upon Sigma calibration) of adults (>18 years) from 13.5 - 34.2 ng/dL to 9.2 - 24.1 ng/dL and for children (<15 years old) 12.2 - 34.9 ng/dL to 9.3 - 22.9 ng/dL.
- Transformed reference intervals were verified using 80 healthy adult specimens and 80 healthy children specimens.

9. Conclusion

- Proper characterization and certification of Reference Materials is critical for use in clinical diagnostic applications. The comparability of materials from various sources demonstrates that unless complete certification is performed, it is not possible to fully evaluate whether a material is suitable for use as a calibrator.
- linearity is an important result in momentous diagnostic use of low reference range materials.
- The data for the LabCorp method was changed to the Cerilliant Certified Spiking Solution® and reference ranges were re-qualified.
- Transformed reference intervals for adults (>16 years) changed from 13.5 - 34.2 ng/dL to 9.2 - 24.1 ng/dL, and for children (1-15 years) from 12.2 - 34.9 ng/dL to 9.3 - 22.9 ng/dL. Transformed reference intervals were verified using specimens from 80 healthy adults and 80 healthy children.