Reverse triiodothyronine (rT3) is an inactive isomer of T3 formed primarily by enzymatic de-iodination of T2, by hydrogenation, followed by terminal ring iodination with iodine and potassium iodide, and purification by reverse phase chromatography.

The availability of accurate reference standards for accurate quantitation of thyroid hormones is essential to have high purity, properly homogenized and well-characterized Reference Materials (RMs) for clinical and analytical applications.

### Synthesis and Purification of T3 and T3

- Commercially available T3 was purchased from Sigma Aldrich and tested at Cerilliant. Material was found to be low purity and lacks therapeutic reference.
- 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 chromatography. Challenges to purification included separation of related impurities T2 and T4, and removal of inorganic content.

### Preparation of Certified Spiking Solutions® of Thyroid Hormones

- Cerilliant synthesized T3 and T3 were certified according to ICH guidelines to produce certified reference materials.
- Spiking solutions were dispensed into amber ampoules, purged with argon, and flame sealed.
- Precautionary process controls were used to ensure accuracy, batch homogeneity and consistency.
- Spiking solutions were certified against an independently-prepared calibration curve.

### Certification of Thyroid Hormone Neat Materials and Spiking Solutions

- rT3 synthesized and tested for use as CRMs by Cerilliant were certified as high purity, free of related contaminants.
- rT3 is expressed as % purity against a Cerilliant-calibrated curve.
- Accurate Reference Standards for Accurate Quantitation of Thyroid Hormones: Impact on Clinical Reference Ranges

### LabCorp Comparison of Thyroid Hormone Reference Materials in a Clinical LC/MS/MS Assay

- 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 30 to 50% high relative to a calibrator prepared in-house from powder T3 obtained from Sigma-Aldrich.
- LabCorp solutions (prepared from Sigma neat material with small weighings) were 9
- Solutions were analyzed in two diluents to rule out diluent effects.
- Solutions were analyzed at 100 µg/mL against a Cerilliant calibration curve by both HPLC/UV and LC/MS/MS.
- The solution purity was evaluated for consistency with the neat material to rule out material variability.
- Accuracy of the prepared concentration was verified by comparison to a independently-prepared check-standard made from Sigma T3 material.
- Mass Balance Purity Factor

### Clinical Reference Ranges: Transformation of Reference Intervals

- The change in calibrator necessitated change in reference materials, transformation and verification.
- A study over multiple days and multiple batches was conducted to obtain a transformation equation for LabCorp current reference intervals using the EPB Adjuster. The resulting slope and intercept difference (0.721 – 0.51) was used to transform the existing reference intervals (based upon Sigma calibration) of adults (18 years) from 13.5 – 34 ng/dL to 22 – 41 ng/dL, and for children (15–19 years) 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.

### Conclusion

- Proper characterization and certification of Reference Materials is critical for use in clinical diagnostic applications. The comparison 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. Insufficient characterization or use of low purity reference materials can result in incorrect therapeutic reference ranges and negatively impact clinical outcomes.
- The calibrator for the LabCorp method was changed to the Cerilliant T3 Certified Spiking Solution® and reference ranges were re-qualified.
- Transformed reference intervals for adults (+6 years) changed from 13.5 – 34 ng/dL to 9.2 – 24.1 ng/dL, and for children (15–19 years) from 12.2 – 34.9 ng/dL, to 8.3 – 22.9 ng/dL. Transformed reference intervals were verified using specimens from 80 healthy adults and 80 healthy children.