High Sensitivity Measurement of Thyroglobulin in Serum in Presence of Anti-Thyroglobulin Autoantibodies

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Materials and Methods

2D-LC Separation
- 1st dimension: Zorbax XDB-CN 50 x 2.1, 5 μm column (Agilent Technologies)
- 2nd dimension: Poroshell 120 FC-C18, 100 x 3.2, 7 μm column (Agilent Technologies)
- Injection volume 50 μL

Evaluation of Assay Performance
- Sensitivity: Analysis of human serum samples containing progressively lower concentration of Tg (range 0.26 – 3.9 ng/mL)
- Linearity: Analysis of seven samples prepared by mixing two serum pools containing 5 ng/mL and 1045 ng/mL Tg in different proportions.
- LOD and LOQ: LOD, limit of detection at which accuracy was within ±15%, precision was <10%, and ratio of the mass transitions maintained within ±30%.
- Dilution integrity: Analyzing patient serum sample containing 3,000 ng/mL of Tg diluted with serum not containing Tg using 5, 10, 20, 30, and 50-fold dilution.
- Ion suppression: post column infusion method.
- Method comparison: Access Beckman-Coulter, DxI 800 using 60 samples tested negative for Tg-AAb and 113 samples tested positive for Tg-AAb.

Standards and Controls
- Calibration standards: 0, 1, 6, 150 ng/mL, Beckman Coulter (Fullertion, CA)
- Quality control samples: pools of human serum samples containing 0, 2, 6, 170 ng/mL of Tg

Results

- Assay is validated.
- Limit of quantitation 0.5 ng/mL (0.76 fmol/mL).
- Injection-to-injection cycle time 6.5 min
- Method is fully validated: LOQ 0.5 ng/mL; LOD 0.25 ng/mL; imprecision <10%.
- Good agreement with Beckman Access immunoassay in samples negative for thyroglobulin autoantibodies.
- In Tg-AAb positive samples tested negative for thyroglobulin using immunassay, LC-MS/MS concentrations were at or above 0.5 ng/mL in 29% of samples.

Conclusions
- Developed quantitative immunoassay LC-MS/MS assay for thyroglobulin.
- Method overcomes interference of endogenous thyroglobulin autoantibodies.
- Accurate measurement of thyroglobulin in presence of thyroglobulin autoantibodies.
- Injection-to-injection cycle time 6.5 min.
- Method is fully validated: LOD 0.5 ng/mL; LOD 0.25 ng/mL; imprecision <10%.
- Good agreement with Beckman Access immunoassay in samples negative for thyroglobulin autoantibodies.
- In Tg-AAb positive samples tested negative for thyroglobulin using immunassay, LC-MS/MS concentrations were at or above 0.5 ng/mL in 29% of samples.

Within-run, between-run and total imprecision of the method

Distribution of Tg concentrations in healthy children and adults

References
- Hooftnagle et al., (Clin Chem, 2008) showed proof of principle for SISCAPA-LC-MS/MS method that overcomes interference of Tg-AAb.
- We present a sensitive and robust SISCAPA-LC-MS/MS method for quantification of Tg in serum and plasma samples that overcomes interference from Tg-AAb.