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# Performance Evaluation of the TSH3-Ultra Assay on the ADVIA Centaur and ADVIA Centaur CP Systems

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## Introduction and Aims

Thyroid-stimulating hormone (TSH) measurements assess thyroid status and aid in the diagnosis and treatment of thyroid disease. High-sensitivity methods defined by NACB guidelines enable the distinction between severely depressed TSH levels seen in Graves' thyrotoxicosis (TSH < 0.01 mIU/L) and TSH levels associated with mild hyperthyroidism (0.01 – 0.1 mIU/L).[1] A functional sensitivity of 0.02 mIU/L (defined as the TSH concentration which yields a coefficient of variation of 20 percent) is accepted as the lowest detection limit (LDL) required to make this distinction as well as the accepted performance criterion for a third-generation TSH assay. The aim of these studies was to validate the performance of the new TSH3-Ultra method on the ADVIA Centaur® and ADVIA Centaur CP Immunoassay Systems.



# Materials and Methods

**Assay:**
The TSH3-Ultra method is a two-site sandwich assay using an FITC-labeled mouse monoclonal anti-human TSH antibody conjugate and an acridinium-labeled Fab anti-human TSH conjugate. The solid phase uses a paramagnetic particle with covalently bound mouse antifluorescein monoclonal antibody (Figure 1).

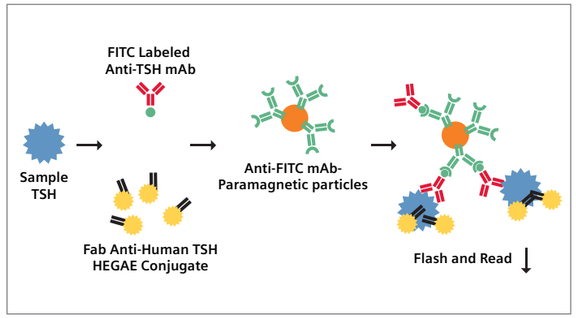


Figure 1

**The Newer Generation Acridinium Ester:**
The TSH3-Ultra assay employs NSP-DMAE-HEG-Glu-NHS (N-Sulfopropyl-Dimethyl Acridinium-Ester Hexa (ethylene) glycol-Glutarate N-hydroxysuccinimide), which is a new generation, high quantum yield hydrophilic acridinium ester label patented (US 6664043) by Siemens Healthcare Diagnostics. NSP-DMAE-HEG-Glu-NHS is significantly more hydrophilic resulting in lower non-specific binding in assays. It also exhibits enhanced light output over DMAE-NHS and greater reactivity for labeling proteins and antibodies (Figure 2).

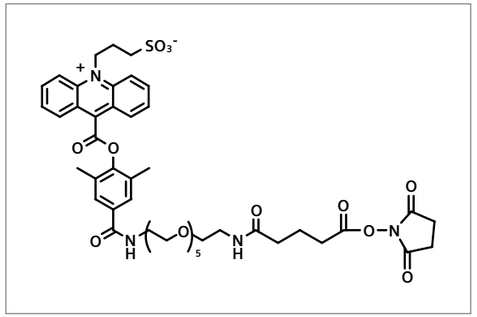


Figure 2

**Samples:**
The TSH3-Ultra method requires only 100 µL of either serum or plasma for a single determination. Samples were obtained from area clinical laboratories.

**Precision:**
Precision was evaluated according to the CLSI protocol EP5-A2.[2] Four controls and six patient serum pools were assayed in duplicate in two runs on both ADVIA Centaur and ADVIA Centaur CP using two reagent lots and two calibrator lots over a 20-day period (n ≥ 80 for each sample).

**Limit of Blank (LoB):**
The Limit of Blank (LoB) was determined as recommended in CLSI guideline EP17-A. A TSH3-Ultra negative equine base pool was used as a blank and was analyzed on two ADVIA Centaur XP instruments and over 12 days and on two ADVIA Centaur CP instruments over 10 days. Each instrument was calibrated on day one only.

**Functional Sensitivity:**
For a third generation TSH assay, researchers agree that the lowest concentration at which the total imprecision does not exceed 20 percent is the functional sensitivity for this assay. For this reason, the functional sensitivity estimate for the TSH3-Ultra assay was determined by using low concentration human serum pools analyzed with two reagent lots on an ADVIA Centaur and an ADVIA Centaur CP System for at least 10 days.

**Dilutional Recovery (Parallelism):**
Six patient samples in the range of 0.296 to 146.24 µIU/mL of TSH were diluted 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 with Multi-Diluent 1, which is an equine-serum-based diluent. TSH was measured at each diluted points.

**Interference Studies:**
The potential interferents hemoglobin, triglycerides, protein (human serum albumin), and bilirubin were added to patient pools and TSH recoveries were determined.

**Expected Results:**
Pediatric, adolescent, and adult reference ranges for the TSH3-Ultra assay were established according to CLSI guideline C28-A2 on the ADVIA Centaur System.[3] Samples from healthy euthyroid individuals were assayed for TSH, FT3, and FT4 and considered normal if their values were within acceptable ranges. Adults were also screened for the presence of thyroid autoantibodies.[4]

## Results

**Precision:**
Precision results are shown in Table 1.

**Limit of Blank:**
The LoB for the ADVIA Centaur and ADVIA Centaur CP Systems are 0.0011 and 0.0049, respectively. Results for LoB are considerably lower than any clinically expected concentrations.

**Functional Sensitivity:**
The functional sensitivity of TSH3-Ultra assay on ADVIA Centaur and ADVIA Centaur CP is 0.008 µIU/mL (mIU/L). It was determined by using multiple patient samples in the range of 0.003 to 0.013 µIU/mL (mIU/L). Based on this study, the TSH3-Ultra assay meets the definition of a third generation TSH assay.[1]

| ADVIA Centaur | | | | |
|---|---|---|---|---|
| | Mean | Within-Run | Run-to-Run | Total |
| Sample | µIU/mL | %CV | %CV | %CV |
| Control 1 | 0.026 | 4.7 | 3.6 | 6.6 |
| Control 2 | 0.383 | 2.0 | 4.1 | 5.1 |
| Control 3 | 4.2 | 2.0 | 4.3 | 5.6 |
| Control 4 | 16.6 | 2.3 | 4.0 | 5.2 |
| Pool 1 | 1.0 | 2.9 | 2.4 | 4.5 |
| Pool 2 | 5.4 | 2.4 | 0.9 | 3.6 |
| Pool 3 | 10.7 | 1.7 | 1.2 | 3.8 |
| Pool 4 | 32.0 | 1.9 | 1.9 | 4.4 |
| Pool 5 | 56.7 | 1.4 | 1.6 | 4.3 |
| Pool 6 | 132.8 | 1.8 | 1.4 | 5.2 |
| **ADVIA Centaur CP** | | | | |
| | Mean | Within-Run | Run-to-Run | Total |
| Sample | µIU/mL | %CV | %CV | %CV |
| Control 1 | 0.025 | 4.1 | 3.0 | 9.5 |
| Control 2 | 0.361 | 2.5 | 3.1 | 4.9 |
| Control 3 | 4.0 | 2.4 | 3.9 | 5.6 |
| Control 4 | 15.7 | 2.9 | 4.4 | 6.0 |
| Pool 1 | 0.9 | 2.1 | 1.9 | 2.9 |
| Pool 2 | 5.0 | 2.1 | 1.6 | 2.7 |
| Pool 3 | 27.0 | 2.5 | 1.7 | 3.3 |
| Pool 4 | 69.9 | 3.8 | 1.5 | 4.2 |
| Pool 5 | 114.4 | 4.4 | 3.8 | 6.3 |
| Pool 6 | 132.2 | 4.9 | 2.7 | 9.9 |

Table 1

**Dilutional Recovery:**
The results for the dilution study are summarized in Table 2.

**Interference:**
Endogenous interfering substances had no effect beyond the limits of random within-run variations on both the ADVIA Centaur and ADVIA Centaur CP Systems. The results demonstrated 10 percent interference from each of the substances tested in the TSH3-Ultra assay (Table 3).

**Expected Results:**
The TSH reference ranges for pediatric, adolescent, and adult samples were determined by calculating the 2.5th and 97.5th percentiles of the distribution of values for each age group below (Table 4).

| | Conc. for Serum Samples (mIU/mL) | Range of Recoveries | Mean Recovery |
|---|---|---|---|
| ADVIA Centaur | 0.296 | 88.4 – 96.6% | 93.6% |
| | 1.674 | 91.6 – 106.1% | 97.0% |
| | 11.72 | 101.8 – 108.3% | 105.9% |
| | 72.26 | 97.8 – 102.6% | 99.6% |
| | 109.39 | 95.7 – 99.8% | 97.0% |
| | 146.24 | 96.3 – 104.4% | 100.3% |
| ADVIA Centaur CP | 11.17 | 101.1 – 113.7% | 96.3% |
| | 41.71 | 97.9 – 110.2% | 100.6% |
| | 69.51 | 95.3 – 102.7% | 98.3% |
| | 114.31 | 86.1 – 95.5% | 90.1% |
| | 126.98 | 85.2 – 95.9% | 89.8% |
| | 147.14 | 89.2 – 99.5% | 93.9% |

Table 2

| Specimens that have: | Demonstrate <10% change in results up to: |
|---|---|
| Hemolysis | 500 mg/dL of hemoglobin |
| Lipemia | 1000 mg/dL of triglycerides |
| Bilirubin | 40 mg/dL of conjugated bilirubin |
| | 40 mg/dL of unconjugated bilirubin |

Table 3

| | Age | Number of Samples | TSH Range (mIU/mL) |
|---|---|---|---|
| Pediatrics | 2 to <12 | 137 | 0.64-6.27 |
| Adolescents | 12 to <18 | 183 | 0.51-4.94 |
| Adults | ≥18 | 229 | 0.55-4.78 |

Table 4


## Conclusions

The ADVIA Centaur TSH3-Ultra assay on the ADVIA Centaur and ADVIA Centaur CP Systems is sensitive and precise, with imprecision performance that exceeds the criteria recommended by the NACB for TSH methods. The enhancements in assay performance result from new solid-phase and conjugate architectures as well as higher affinity anti-human TSH antibodies and use of a proprietary acridinium ester. These innovations have allowed a higher TSH sensitivity detection, wider liner range, and a reduction in sample volume to 100 µL.

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