

Evaluation of the ADVIA®Centaur™ TSH-3 Assay

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An analytical evaluation of the thyroid stimulating hormone (TSH-3) assay on the Bayer ADVIA® Centaur™ immunoassay system was performed. General analytical requirements (linearity, resistance to typical interferences, absence of a carry-over effect) were fulfilled and reproducibility was satisfactory. Inter-assay coefficient of variation (CV) of a human serum pool with a concentration of 0.014 mU/l was 22.3%; at concentrations between 0.26 and 83 mU/l CV was below 6%. Method comparison study demonstrated close agreement of TSH results compared to those obtained with the Roche Elecsys® 2010 TSH assay (ADVIA Centaur = 1.08 x Elecsys – 0.18 mU/l; $r=0.987$; $n=324$). Handling and practicability of the ADVIA Centaur system proved to be convenient with a very high sample throughput. We conclude that the ADVIA Centaur TSH-3 assay meets requirements for clinical use.

Key words: TSH (thyrotropin); Immunoassay; Automation; ADVIA® Centaur™.

Abbreviations: CV, coefficient of variation; TSH, thyroid stimulating hormone (thyrotropin).

Introduction

Thyrotropin (TSH) is now generally accepted as the first line parameter in the screening for thyroid diseases (1). Since the prevalence of thyroid diseases is high in the general population, especially in iodine deficient regions (2), and symptoms are rather unspecific in many cases, screening for thyroid disorders is indicated in a large proportion of patients requiring medical attention. Therefore TSH assays represent one of the highest volume immunoassays in many clinical laboratories. Consequently, reliable automation is of particular importance for TSH determinations.

The third generation TSH assay implemented on the Bayer ACS:180® immunoassay analyzer has been widely used for several years now and evaluation data has been published previously (3–6). This TSH assay is now available on the new ADVIA® Centaur™ immunoanalyzer (formerly designated as the ACS:Centaur® immunoanalyzer) with the added feature of an exceptionally high sample throughput. The aim of our study was to investigate whether the rapid throughput of the Bayer ADVIA® Centaur™ TSH-3 assay maintained acceptable assay performance.

Materials and Methods

Instruments

The ADVIA Centaur analyzer system (Bayer Diagnostics, Tarrytown, NY, formerly Chiron Diagnostics, East Walpole, MA, USA) is a stand alone analyzer providing the features that are characteristic for modern automated immunoassay systems: random-access and multichannel working mode, calibration stability, positive sample identification by a bar-code reader, and bi-directional interface to a laboratory software system. Sample tubes are released within three minutes after pipetting for further processing. All reagents and supply materials (water, cuvettes, pipette tips) may be refilled and waste may be emptied during a run. Reagents are mixed manually before loading and can then remain on-board the assay system for 28 days. Both assay cuvettes and pipette-tips are disposable single use materials. The system is equipped with a clot-detection device. Daily maintenance consists of an automated washing procedure that takes about 40 minutes and the routine start-up takes about 10 minutes. The maximum throughput is 240 samples per hour. The analyzer requires an area of approximately 2.5 square meters for installation.

Assay characteristics

The ADVIA Centaur TSH-3 assay (Bayer Diagnostics) is a sandwich immunoassay using paramagnetic microparticles as solid phase and direct chemiluminescence of acridinium ester for detection of specific signal. Two hundred microliters (ml) of sample are dispensed in a cuvette, and 100 μ l of a buffered solution of a monoclonal anti-TSH antibody labeled with an acridinium ester are added and incubated at 37°C for 2.5 minutes. A homogenized suspension of the paramagnetic microparticles (225 μ l) coated with a polyclonal sheep-anti-TSH antibody is then added; incubation for 5 minutes allows formation of the sandwich. The microparticles are separated with magnets from the solution and washed. Acid and base reagent additions trigger the chemiluminescent reaction, that is detected by a photomultiplier. The intensity of the light signal measured over 5 seconds is directly related to the TSH concentration in the sample. A predefined master calibration curve of the respective reagent lot is read from the reagent cartridges by a barcode scanner. This master curve is adjusted by the customer using two calibrators when a new reagent lot is first introduced and after a recommended interval of 28 days. The assay is standardized according to the international standard preparation 2nd IRP 80/558 (WHO). The measuring range of the assay is from 0.004 mU/l (analytical sensitivity) to 150 mU/l. Results are reported approximately 15 minutes after pipetting the sample. The reference range recommended by Bayer Germany for the ADVIA Centaur TSH-3 assay is 0.35–4.5 mU/l.

Assay studies

Imprecision study

Human serum pools at five concentration levels were prepared from serum samples, aliquoted after overnight equilibration, and stored at –20°C. Lyophilized control materials at

three concentration levels (Ligand Plus 1,2,3 Quality Control material; Bayer Diagnostics) were reconstituted with deionized water and used within three days thereafter. Intra-assay imprecision studies in 21 replicates were performed using the human serum pools. To study inter-assay imprecision the aliquoted human serum pools and the control materials were analyzed in 21 runs over a 35-day period in singlicates; two different lots of reagents and calibrators were used. Recalibration was performed every two weeks.

Carry-over study

A sample of a patient with hyperthyroidism was measured in five replicates directly after five replicates of a sample with a high TSH concentration.

Linearity study

A high-TSH human serum pool (91 mU/l) was diluted in nine steps with a low-TSH human serum pool (0.008 mU/l) (9+1, 8+2, ... 2+8, 1+9 volumes of the high and the low pool, respectively). TSH concentrations of the diluted samples were determined after two hours of equilibration; Pearson's correlation coefficient was calculated for the results obtained from the eleven samples. Linearity in the lower measuring range was tested by diluting a serum pool with a concentration of 1.9 mU/l with the low-TSH pool using the same dilution sequence.

Interference studies

Possible interference with the assay from sera of patients with uremia, cholestasis, lipemia or heterophilic antibodies was assessed by dilution experiments. The same nine-step dilution sequence as used for the linearity investigations was applied using four high-interferent pools (lipemia, triglycerides 11.5 mmol/l; uremia, creatinine 778 μ mol/l; icterus, bilirubin 231 μ mol/l; heterophilic antibodies, 65 IU/ml (N Latex RF, nephelometric assay, Dade Behring, Marburg, Germany) and one normal pool, respectively, the latter with a TSH concentration of 0.14 mU/l. Pearson's coefficient of correlation was calculated for the four respective studies; if linearity of TSH results was found in the dilution series ($r > 0.98$) absence of relevant interference by the respective factors in the given concentrations was assumed. To study the influence of hemolysis, 2 ml of a human serum pool were spiked with 10 μ l of a hemolysate obtained by freezing a whole blood sample, resulting in a free hemoglobin concentration of 2.9 μ mol/l; TSH was measured in triplicate before and after spiking, respectively.

Method comparison

Serum samples for TSH determination obtained over a one month period by the Clinic of Nuclear Medicine of our institution were used for a method comparison study. Included in this study were 32 patients with overt hyperthyroidism before and during radioiodine therapy, 28 patients during thyroxine withdrawal before undergoing radioiodine therapy for differentiated thyroid carcinoma, and 61 patients under long term thyroxine substitution after treatment for thyroid carcinoma. Additionally 265 routine consecutive samples sent for TSH determination were included in the method comparison (168 outpatients, 97 hospitalized patients including 58 intensive care patients); a total of 389 samples from 380 patients was studied.

The results of the ADVIA Centaur TSH-3 assay were compared with those obtained with our current routine method, the Roche Elecsys® 2010 TSH assay (Roche Diagnostic Systems, Mannheim, Germany); this immunoassay system uses microparticles as solid phase as well, but electrochemiluminescence as principle of signal generation. The Elecsys TSH assay, a sandwich assay with two monoclonal antibodies (standardized according to the IRP WHO standard preparation 80/558; sample volume 50 μ l) has been evaluated previously (7). Single measurements were performed within four hours on both assay systems. Samples with a TSH concentration above 100 mU/l found with the Elecsys assay were not included in the study. The results of the two assays were analyzed by linear regression. Additionally the mean difference between the two assays was calculated as described by Bland and Altman (8).

All handling instructions of the manufacturer were followed in all regards and quality control was performed in each run according to the guidelines of the German Medical Association by use of three commercial control materials (see above).

Results

In the imprecision study, an inter-assay coefficient of variation (CV) of 22.3% was obtained in a human serum pool with a TSH concentration found at 0.014 mU/l. For the other pools and the control materials CVs below 6% were found (Table 1).

No carry-over effect was detected. After five determinations of TSH in a sample with a concentration of 83 mU/l, five consecutive results of low-TSH sample were as follows: 0.025, 0.029, 0.029, 0.027, and 0.026 mU/l.

Tab. 1 Imprecision study of the ADVIA Centaur TSH-3 assay.

Sample	Mean TSH concentration (mU/l)	Intra-assay imprecision (CV%) n=21	Inter-assay imprecision (CV%) n=21
Pool 1	0.014	16.7%	22.3%
Pool 2	0.26	3.9%	5.5%
Control Ligand 1	0.51		5.5%
Pool 3	0.98	2.4%	5.1%
Control Ligand 2	4.38		5.8%
Pool 5	6.80	2.9%	3.1%
Control Ligand 3	16.4		5.5%
Pool 6	83.2	2.8%	4.4%

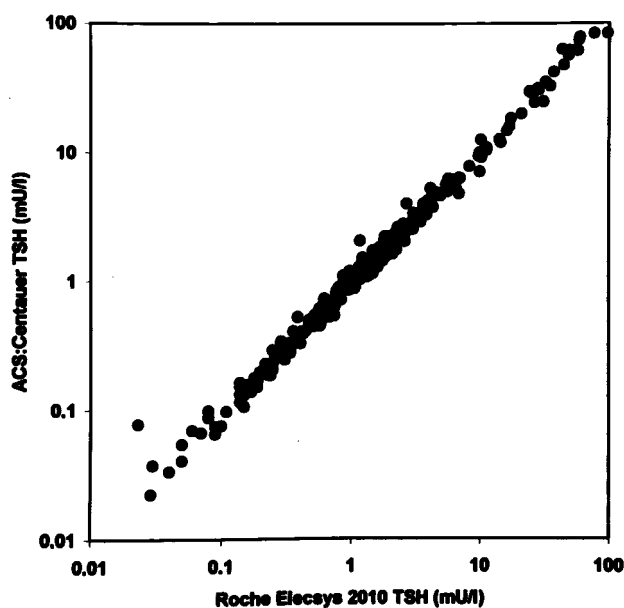


Fig. 1 Method comparison study between the ADVIA Centaur TSH-3 assay and the Roche Elecsys 2010 TSH assay (note logarithmic scales; $n=324$; linear regression analysis for all paired results: $\text{ADVIA Centaur TSH} = 1.08 \times \text{Elecsys TSH} - 0.18 \text{ mU/l}$; $r=0.987$).

In the dilution experiments, linearity of the TSH results was demonstrated in the studied range up to 91 mU/l through a correlation coefficient of 0.998. The assay proved linear in the low measuring area ($< 1.9 \text{ mU/l}$) as well ($r = 0.998$).

No analytical interference from uremia, cholestasis, and heterophilic antibodies was found in our experiments, with correlation coefficients >0.98 for each of the dilution experiments. Marked hemolysis led to slightly lower results; in a sample with a TSH concentration of 2.37 mU/l, spiking with hemolysate leading to free hemoglobin concentrations of 2.0 and 5.0 g/l, respectively, caused a decrease of TSH results by -3.6% and -8.8% .

In the method comparison study, 324 results within the range of 0.02 mU/l (which was assumed as functional sensitivity of the assays) and 100 mU/l were found (see Figure 1).

The mean concentration of these samples was 4.9 mU/l (SD 11.6, median 1.2) with the ADVIA Centaur assay, and 4.7 mU/l (SD 12.7, median 1.2) with the Elecsys assay. The mean percentage difference between the two assays (ADVIA Centaur results subtracted from the respective Elecsys results, according to Bland and Altman (7)) was $+3.8\%$ (SD 14.2%). Linear regression analysis revealed the following equations for the different concentration ranges:

0.02–100 mU/l: $\text{ADVIA Centaur} = 1.08 \times \text{Elecsys} - 0.18 \text{ mU/l}$ ($n=324$; $r=0.987$)

0.02–1.0 mU/l: $\text{ADVIA Centaur} = 0.93 \times \text{Elecsys} + 0.004 \text{ mU/l}$ ($n=127$; $r=0.982$)

Among the population studied, 65 patients had very low TSH concentrations. In 61 sample both assays measured concentrations below 0.02 mU/l; in four

samples one of the assays found a concentration below 0.02 mU/l, the other showed slightly higher results (TSH in mU/l: sample 1, Elecsys 0.023, Centaur 0.019; sample 2, Elecsys 0.012, Centaur 0.023; sample 3, Elecsys 0.016, Centaur 0.027; sample 4, Elecsys 0.018, Centaur 0.031). The most marked relative difference between Centaur and Elecsys TSH results (0.08 mU/l, and 0.02 mU/l, respectively) was found in a sample from a surgical intensive care patient; both serum free thyroxine and triiodothyronine were low, representing the characteristic constellation seen in severe non-thyroidal illness.

The test throughput of 240 determinations per hour specified for the ADVIA Centaur could be verified in our experiments.

Discussion

The ADVIA Centaur TSH-3 assay demonstrated satisfactory precision in our experiments and fulfilled the criteria of a third generation TSH assay (9, 10). Long-term stability of the assay results will depend on production quality of all following lots and will be assessed prospectively. Similar imprecision data have been reported for automated chemiluminescence assays (4, 5, 11).

Further general analytical requirements of the ADVIA Centaur TSH-3 assay could be verified: dilution linearity was found to be satisfactory over the whole range, as well as in the low measuring range, and no carry-over effect was detected. The assay was unaffected by typical assay interferences, except for marked hemolysis.

The ADVIA Centaur TSH results were in very close agreement to those obtained with our current routine method in a large number of clinical samples from patients with various thyroid disorders over a wide concentration range; even in the very low range the diagnostic agreement of both assays was excellent.

The high sample volume (200 μl) necessary for a TSH determination may limit the use of the ADVIA Centaur TSH-3 assay in pediatric medicine.

The high sample throughput of the ADVIA Centaur analyzer, 240 determinations per hour as specified by the manufacturer, was indeed realized during our evaluation. Reagent and disposal handling was easy and convenient. The same applies for the system software. Our experiences with regard to practicality were satisfactory.

In summary, we conclude from our evaluation data that the ADVIA Centaur TSH-3 assay meets the analytical quality requirements for its routine clinical use as well as for a high sample throughput.

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