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ADVIA Centaur® XP
ADVIA Centaur® XPT
Immunoassay Systems

anti-Tg (aTG)

Assay for the Detection of Autoantibodies against Thyroglobulin

Current revision and date [a]	Rev. D, 2020-05	
Product Name	ADVIA Centaur® anti-Tg assay (500 tests) ADVIA Centaur anti-Tg assay (100 tests)	**REF** 10492399 **REF** 10492398
Systems	ADVIA Centaur XP system ADVIA Centaur XPT system	
Materials Required but Not Provided	ADVIA Centaur Calibrator 1	**REF** 10630915
Specimen Types	Serum, EDTA Plasma	
Assay Range	15–500 U/mL	
Reagent Storage	2–8°C	
Reagent On-System Stability	28 days	

a In Rev. B or later, a vertical bar in the margin indicates a technical update to the previous version.

Intended Use

For *in vitro* diagnostic use in the quantitative determination of autoantibodies against thyroglobulin in serum or EDTA plasma using the ADVIA Centaur® XP and ADVIA Centaur® XPT systems. This assay is intended to be used as an aid in the diagnosis of Hashimoto's and Graves' diseases which are autoimmune diseases affecting the thyroid gland.

Summary and Explanation

Thyroglobulin (Tg) is a large, heterogeneous glycoprotein (MW 660,000) found in the follicular cells of the thyroid. Thyroglobulin plays an important role in the biosynthesis of thyroid hormones, T_3 and T_4. In the thyroid follicular cells, thyroid peroxidase catalyzes the iodination of tyrosyl groups within thyroglobulin. Iodinated thyroglobulin is stored in the colloid of the follicle and serves as a storage reservoir for T_3 and T_4. When the thyroid gland is stimulated, thyroglobulin is degraded and the thyroid hormones, T_3 and T_4, are released into the bloodstream.[1–3]

The measurement of autoantibodies against thyroglobulin is useful in identifying patients with autoimmune thyroid disease. Levels of anti-Tg antibodies are increased in 80 to 100% of patients with Hashimoto's or chronic thyroiditis, 60 to 70% of patients with Graves' disease, and in 10 to 20% of patients with subacute thyroiditis. Because of the heterogeneity of thyroglobulin, anti-thyroglobulin antibodies have been detected in other disease states, in elderly patients and also in clinically normal, euthyroid patients. Anti-Tg antibodies have been detected in patients with idiopathic Addison's disease and in some patients with Type I diabetes mellitus.[2,4,5]

Principles of the Procedure

The ADVIA Centaur anti-Tg assay is a competitive immunoassay using direct, chemiluminescent technology. Autoantibody against thyroglobulin in the patient sample competes with polyclonal human anti-Tg antibody bound to polyclonal goat anti-human antibody covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of acridinium ester-labeled human thyroglobulin in the Lite Reagent.

Reagents

Reagent	Description	Storage	Reagent Stability
ADVIA Centaur aTG ReadyPack® primary reagent pack; Lite Reagent	10.0 mL/reagent pack human thyroglobulin (~ 0.38 µg/mL) labeled with acridinium ester in buffer with BSA, sodium azide (< 0.1%), protein stabilizers, and preservatives	2–8°C	**Unopened:** Stable until the expiration date on the carton **On-system:** 28 days
ADVIA Centaur aTG ReadyPack primary reagent pack; Solid Phase Reagent	20.0 mL/reagent pack polyclonal human anti-Tg antibody (~ 1.98 µg/mL) bound to polyclonal goat anti-human antibody (~ 49.5 µg/mL) covalently coupled to paramagnetic particles in buffer with BSA, sodium azide (< 0.1%), and preservatives	2–8°C	**Unopened:** Stable until the expiration date on the carton **On-system:** 28 days
ADVIA Centaur ReadyPack ancillary reagent pack; aTG Diluent[a] DIL	5.0 mL/reagent pack human plasma with sodium azide (< 0.1%) and preservatives	2–8°C	**Unopened**: Until the expiration date on the pack **On-system**: 14 consecutive days after accessing the ancillary reagent pack
ADVIA Centaur aTG Diluent[a] DIL	10.0 mL/vial human plasma with sodium azide (< 0.1%) and preservatives	2–8°C	**Unopened**: Until the expiration date on the vial

a See *Optional Materials*

Warnings and Precautions

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics.

CAUTION POTENTIAL BIOHAZARD

Contains human source material. While each human serum or plasma donor unit used in the manufacture of this product was tested by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2, all products manufactured using human source material should be handled as potentially infectious. Because no test method can offer complete assurance that hepatitis B or C viruses, HIV, or other infectious agents are absent, these products should be handled according to established good laboratory practices.[6–8]

CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

For *in vitro* diagnostic use.

Preparing Reagents

All reagents are liquid and ready to use.

Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to the system operating instructions.

Note

- Discard the primary reagent packs at the end of the on-system stability interval.
- Do not use reagents beyond the expiration date.

Storing and Stability

Store the reagents upright at 2–8°C.

Protect reagent packs from all heat and light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2–8°C away from heat and light sources.

All reagents are stable at 2–8°C until the expiration date on the packaging.

Specimen Collection and Handling

Serum or EDTA plasma are the recommended sample types for this assay.

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI):[9]

- Collect all blood samples observing universal precautions for venipuncture.
- Allow samples to clot adequately before centrifugation.
- Centrifuge samples at ≥ 1000 x g for 15–20 minutes.
- Keep tubes stoppered and upright at all times.
- Do not use samples that have been stored at room temperature for longer than 8 hours.
- Separate serum or plasma from the red blood cells before storage at 2–8°C or -20°C.
- Tightly cap and refrigerate specimens at 2–8°C if the assay is not completed within 8 hours.
- Freeze samples at or below -20°C if the sample is not assayed within 48 hours.
- Freeze samples only once and mix thoroughly after thawing.

The purpose of handling and storage information is to provide guidance to users. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Procedure

Materials Provided

The following materials are provided:

REF	Contents	Number of Tests
10492399	5 ReadyPack primary reagent packs containing ADVIA Centaur aTG Lite Reagent and Solid Phase	500
	ADVIA Centaur aTG Master Curve card	
10492398	1 ReadyPack primary reagent packs containing ADVIA Centaur aTG Lite Reagent and Solid Phase	100
	ADVIA Centaur aTG Master Curve card	

Materials Required but Not Provided

The following materials are required to perform this assay, but are not provided:

Item	Description	
REF 10630915	ADVIA Centaur Calibrator 1	2 vials of low calibrator CAL L 2 vials of high calibrator CAL H

Optional Materials

The following materials may be used to perform this assay, but are not provided:

Item	Description	
REF 07656295 (128281)	ADVIA Centaur aTG Diluent DIL	6 ReadyPack ancillary reagent packs containing 5 mL/pack
REF 09348261 (128280)	ADVIA Centaur aTG Diluent DIL	2 ReadyPack ancillary reagent packs containing 5 mL/pack
REF 00994179 (127636)	ADVIA Centaur aTG Diluent DIL	10 mL/vial
REF 10492692	ADVIA Centaur aTG Master Curve Material	6 x 1 mL
REF 10630917	ADVIA Centaur aTG 1, 2 Quality Control Material	3 x 2 mL control 1 CONTROL 1 3 x 2 mL control 1 CONTROL 2

Assay Procedure

For detailed instructions on performing the procedure, refer to the system operating instructions.

The system automatically performs the following actions:

- Dispenses 40 µL of sample into a cuvette.
- Dispenses 100 µL of Lite Reagent and incubates for 2.5 minutes at 37°C.
- Dispenses 200 µL of Solid Phase and incubates for 5.0 minutes at 37°C.
- Separates, aspirates, and washes the cuvettes with reagent water.

  Note For information about reagent water, refer to the system operating instructions.
- Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
- Reports results according to the selected option, as described in the system operating instructions.

An inverse relationship exists between the amount of anti-Tg present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Preparing the System

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions.

Load the ReadyPack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. For detailed information about loading reagents, refer to the system operating instructions.

If automatic dilution of a sample is required, load ADVIA Centaur anti-Tg Diluent in the ancillary reagent entry.

Preparing the Samples

This assay requires 40 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to the system operating instructions.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation at ≥ 1000 x g for 15–20 minutes.
- Samples are free of bubbles.

On-System Stability

The ADVIA Centaur aTG assay reagents are stable unopened until the expiration date on the carton or onboard the system for 28 days.

Performing Calibration

For calibration of the ADVIA Centaur aTG assay, use the ADVIA Centaur Calibrator 1. Perform that calibration as described in the calibrator instructions for use.

Calibration Frequency

Calibrate the assay at the end of the 14-day calibration interval.

Additionally, the ADVIA Centaur anti-Tg assay requires a two-point calibration:

- When changing lot numbers of primary reagent packs.
- When replacing system components.
- When quality control results are repeatedly out of range.

Performing Master Curve Calibration

The ADVIA Centaur anti-Tg assay requires a Master Curve calibration when using a new lot number of Lite Reagent and Solid Phase. For each new lot number of Lite Reagent and Solid Phase, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions.

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

For detailed information about entering quality control values, refer to the system operating instructions.

To monitor system performance and chart trends, as a minimum requirement, two levels of quality control material should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

For quality control of the ADVIA Centaur anti-Tg assay, use anti-Tg Control or an equivalent quality control material. Refer to the quality control product insert for the suggested Expected Values.

Taking Corrective Action

If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

Results

Calculation of Results

For detailed information about how the system calculates results, refer to the system operating instructions.

The system reports anti-Tg results in U/mL.

Dilutions

The following information pertains to dilutions:

- Patient samples with anti-Tg levels greater than 500 U/mL must be diluted and retested to obtain accurate results.
- Due to the nature of antibody assays, samples may not dilute in a linear manner.
- Patient samples can be automatically diluted by the system or prepared manually.
- For automatic dilutions, ensure that ADVIA Centaur anti-Tg Diluent is loaded and set the system parameters as follows:

  Dilution point: ≤ 500 U/mL

  Dilution factor: 5

  For detailed information about automatic dilutions, refer to the system operating instructions.
- Manually dilute the patient samples when patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.
- Use anti-Tg Diluent to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.
- Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result.

Interpretation of Results

Results of this assay should always be interpreted in conjunction with patient's medical history, clinical presentation and other findings.

Limitations

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[10] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Performance of this assay has not been established with neonatal specimens.

Expected Values

In the following categories 581 patients were analyzed using the ACS:180® anti-Tg reagents. A value of 60 U/mL has been established as a cut-off to differentiate between anti-Tg positive and anti-Tg negative.

Sample Category	N	Median (U/mL)	Mean (U/mL)	% > 60 U/mL
Euthyroid	293	10	22	10
Hashimoto's Disease	189	95	104	70
Graves' Disease	99	21	69	39

These results were confirmed for the ADVIA Centaur anti-Tg assay. Refer to *Accuracy / Method Comparison*.

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[11]

Performance Characteristics

Analytical Measuring Range

The ADVIA Centaur aTG assay measures autoantibodies against thyroglobulin concentrations from 15–500 U/mL.

Specificity

The cross-reactivity of the ADVIA Centaur anti-Tg assay was determined by spiking serum samples with the following compounds at the indicated levels. These compounds did not have a significant effect on the anti-Tg measurement.

Compound	Amount Added	% Crossreactivity
Thyroglobulin	19,000 ng/mL	-0.41
T_3 antibodies	550 µg/mL	-1.16
T_4 antibodies	1010 µg/mL	-0.51

Interference testing was determined according to CLSI Document EP7-A2.[12]

Sensitivity

The ADVIA Centaur anti-Tg assay measures anti-Tg concentrations up to 500 U/mL with a limit of blank (LoB) of 8 and a limit of detection (LoD) of 15.

The LoB is defined as the concentration of anti-Tg that corresponds to the 95th percentile of the distribution of the anti-Tg zero standard or diluent. The anti-Tg zero standard or diluent was assayed 10 times in at least 3 runs each using 2 lots of reagents on 2 systems (n = 120) over a 3-day period.

The LoD is defined as the lowest concentration of anti-Tg that can be detected with 95% probability. The LoD was determined by running 5 low-level anti-Tg serum samples that were assayed 2 times in at least 3 runs each using 2 lots of reagents in 2 systems (n = 120) over a 3-day period.

The LoB and LoD were determined as described in the CLSI Document EP17-A.[13]

Precision

Three samples were assayed 6 times in 15 runs, on at least 6 systems (n = 90 for each sample). The following results were obtained:

Mean anti-Tg (U/mL)	Within-run % CV	Run-to-run % CV	Total % CV
71	5.8	2.1	6.2
170	3.7	1.2	3.9
344	3.4	5.7	6.6

Accuracy / Method Comparison

For 255 serum samples in the range of 10 to 499 U/mL, the relationship between the ADVIA Centaur anti-Tg assay and the ACS:180 anti-Tg assay is described by the equation:

ADVIA Centaur anti-Tg = 1.03 (ACS:180 anti-Tg) + 2.29 U/mL

Correlation coefficient (r) = 0.98

The diagnostic concordance between the two assays is 98.0% (250/255). The following results were obtained:

Category	ACS:180 Positive	ACS:180 Negative
ADVIA Centaur Positive	110	4
ADVIA Centaur Negative	1	140

Interferences

Serum specimens that are . . .	Demonstrate ≤ 5% change in results up to . . .
hemolyzed	100 mg/dL of hemoglobin
lipemic	1000 mg/dL of triglycerides
icteric	40 mg/dL of bilirubin
proteinemic	12 g/dL of protein

Dilution Recovery

Five human serum samples in the range of 391 to 542 U/mL of anti-Tg were serially diluted 1:2, 1:4, and 1:8 with anti-Tg Diluent and assayed for recovery and parallelism. The recoveries ranged from 80.4% to 111.7% with a mean of 95.3%.

Sample	Dilution	Observed (U/mL)	Expected (U/mL)	Recovery %
1	—	404		
	1:2	200	202	99.2
	1:4	99.7	101	98.7
	1:8	54.5	50.5	107.9
	Mean			102.0
2	—	391		
	1:2	167	195	85.7
	1:4	86.7	97.7	88.8
	1:8	54.6	48.8	111.7
	Mean			95.4
3	—	471		
	1:2	222	235	94.3
	1:4	115	118	97.8
	1:8	62.4	58.9	106.0
	Mean			99.4
4	—	531		
	1:2	213	265	80.4
	1:4	113	133	84.9
	1:8	61.2	66.3	92.2
	Mean			85.8
5	—	542		
	1:2	236	271	87.2
	1:4	126	135	92.9
	1:8	68.6	67.7	101.3
	Mean			93.8
Mean				**95.3**

Spiking Recovery

Varying amounts of anti-Tg were added to 5 serum sample with endogenous anti-Tg levels of 15–53 U/mL. The amount of anti-Tg that was added varied from 138–405 U/mL. When compared to the expected value, the measured (recovered) values of anti-Tg averaged 94.7% with a range of 87.3% to 102.3%.

Sample	Amount Added (U/mL)	Observed (U/mL)	Expected (U/mL)	Recovery %
1	—	28		
	138	145	166	87.3
	332	344	360	95.5
	405	407	433	94.0
	Mean			92.3
2	—	15		
	138	135	153	88.2
	332	334	347	96.3
	405	418	420	99.5
	Mean			94.7
3	—	53		
	138	172	191	90.1
	332	361	385	93.8
	405	442	458	96.5
	Mean			93.5
4	—	25		
	138	146	163	89.6
	332	348	357	97.5
	405	440	430	102.3
	Mean			96.5
5	—	47		
	138	170	185	91.9
	332	376	379	99.2
	405	447	452	98.9
	Mean			96.7
Mean				**94.7**

Standardization

The ADVIA Centaur anti-Tg assay standardization is maintained with internal standards manufactured using human autoantibodies against thyroglobulin. The ADVIA Centaur anti-Tg standardization is traceable to World Health Organization (WHO) Reference Preparation MRC 65/93. The theoretical WHO International units (IU/mL) average 3-fold higher compared to Siemens Healthcare Diagnostics standardization. Assigned calibrator doses and ranges for quality control material are traceable to this standardization.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

www.siemens.com/diagnostics

References

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8. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030.
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10. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem.* 1988;34:27–33.
11. Clinical and Laboratory Standards Institute (formerly NCCLS). *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2000. NCCLS Document C28-A2.
12. Clinical and Laboratory Standards Institute (formerly NCCLS). *Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI Document EP7-A2.
13. Clinical and Laboratory Standards Institute (formerly NCCLS). *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP17-A.

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	REF REF	Catalog number
	Legal manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Mark	CE 0088	CE Mark with identification number of notified body
	Consult instructions for use		Biological risk
0°C	Do not freeze (> 0°C)		Temperature limitation
	Lower limit of temperature		Upper limit of temperature
	Keep away from sunlight and heat	UP	Up
	Use by	Σ (n)	Contains sufficient for (n) tests
LOT	Batch code		Shake the reagent pack vigorously. Refer to *Preparing Reagents* in the assay-specific ADVIA Centaur product instructions for detailed information.
YYYY-MM-DD	Date format (year-month-day)	Rev.	Revision
MC DEF	Master Curve Definition	CHECKSUM	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.
LOT DTL	Lot Details	DER GRÜNE PUNKT	Green dot
	Recycle	PRINTED WITH SOY INK	Printed with soy ink

Trademarks

US Pats 5,609,822; 5,788,928

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