T3, Total
Triiodothyronine (T3), Total
ADVIA Centaur
By Direct Chemiluminescence

I. Principle
The ADVIA Centaur T3 assay is a competitive immunoassay using direct chemiluminescent technology. T_3 in the patient sample competes with a T_3 analog, which is covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of acridinium ester-labeled monoclonal mouse anti-T_3 antibody in the Lite Reagent. An inverse relationship exists between the amount of T_3 present in the patient sample and the amount of relative light units (RLUs) detected by the system.

II. Clinical Significance
Triiodothyronine (3,5,3'-L-triiodothyronine, T3) is a hormone that originates from direct thyroid synthesis and secretion (approximately 20%) and from peripheral conversion of T_4 to T_3 (approximately 80%). T_3 is secreted into the circulation in response to the pituitary hormone TSH (thyroid stimulating hormone). The secretion of T_3 is regulated by a negative feedback mechanism involving the thyroid gland, pituitary gland, and hypothalamus. Although serum levels of T_3 are small, it has greater physiological potency than T_4.

In the circulation, 99.7% of T_3 is reversibly bound to transport proteins, primarily thyroxine binding globulin (TBG) and to a lesser extent albumin and thyroxine binding prealbumin (TBPA). Unbound or free T_3 is metabolically active and bound T_3 is metabolically inactive, acting as a reserve for free T_3.

TBG concentrations remain relatively constant in healthy individuals. However, pregnancy, excess estrogens, androgens, anabolic steroids, and glucocorticoids are known to alter TBG levels and may cause false thyroid values for thyroid function tests. T_3 levels in these situations may not accurately reflect thyroid status.

Primary malfunction of the thyroid gland may result in excessive (hyper) or below normal (hypo) release of T3 or T4. In addition, as thyroid function is directly affected by TSH, malfunction of the pituitary or the hypothalamus influences the thyroid gland activity. Disease in any portion of the thyroid-pituitary-hypothalamus system may influence the levels of T3 and T4 in the blood.

Diagnostically, T3 concentration is more sensitive to certain thyroid conditions than T4. While T4 levels are a sensitive (and superior) indicator of hypothyroidism, T3 blood levels better define hyperthyroidism.

Because T3 concentration in serum changes faster and more markedly than T4, the T3 level is also an excellent indicator of the ability of the thyroid to respond to both stimulatory and suppressive tests. Under conditions of strong thyroid stimulation, the T3 level offers a good estimation of thyroidal reserve as well.

III. Specimen

Serum is the recommended sample type for this assay.

A. Collect: One SST or red top. (Min: 6 mL)

B. Transport: SST, or 1 mL serum (Min: 185 ul) at ambient or 2 – 8°C (see stability).

C. Unacceptable Conditions:

Plasma samples
Gross hemolysis
Samples that have been stored at room temperature for ≥ 8 hours

D. Stability: Ambient 20-25°C 8 hours; 2-8°C: 48 hours; <-20°C: 3 months

Store samples at room temperature for no longer than 8 hours. Freeze samples only once and mix thoroughly after thawing.

IV. Reagents

Reagent	*Volume*	*Ingredients*
Lite Reagent	8.0 mL/reagent pack	monoclonal mouse anti-T_3 antibody (~60 ng/mL) labeled with acridinium ester in buffered saline with sodium azide (0.1%), sodium barbital, and ANS
Solid Phase	24.0 mL/reagent pack	T_3 analog (~13.3 µg/mL) covalently coupled to paramagnetic particles in HEPES buffer with sodium azide (0.1%), sodium barbital, and ANS
T3/T4/VB12 Ancillary Reagent	25.0 mL/reagent pack	0.4N sodium hydroxide
T3 Diluent	10 mL/vial	human plasma and sodium azide (0.1 %)

A. Precautions

1. **NOTE:** Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.
2. **HARMFUL:** Harmful if swallowed. After contact with skin, wash immediately with plenty of soap and water. Contains: sodium azide; Lite Reagent, Solid Phase.
3. **IRRITANT:** Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. Contains: sodium hydroxide; Ancillary Reagent.

4. **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.
5. **CAUTION:** This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

B. Storage and Stability:

1. Store the reagents upright at 2 – 8°C.
2. Lite Reagent and Solid Phase stable until the expiration date on the pack label, or for 28 days onboard the system.
3. T3/T4/VB12 Ancillary Reagent stable until the expiration date on the pack label, or for 14 consecutive days after accessing the ancillary reagent pack.
4. T3 Diluent stable until the expiration date on the vial label.
5. Discard the primary reagent packs at the end of the onboard stability interval.
6. Do not use reagents beyond the expiration date.

C. Loading Reagents

1. Ensure that the system has sufficient primary and ancillary reagent packs.
2. **CAUTION:** 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 re-suspended.
3. Load the Ready Pack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogenous suspension of the reagents.
4. Load the T3/T4/B12 Ancillary Reagent ReadyPack in the ancillary reagent entry.

V. Instrumentation/Equipment

The ADVIA Centaur and ADVIA Centaur XP systems are automated, direct chemiluminescent immunoassay analyzers that offer optimal productivity and efficiency. No-pause reloading of reagents, samples, and supplies means that the system is always ready to process samples.

Sample racks are loaded in the sample entry queue and the sample start button is pressed to activate the test sequence. The sample entry queue moves the sample racks to the inprocess queue, where the sample is aspirated and dispensed it into a cuvette in the incubation ring.

Reagents are dispensed into the cuvette, the reaction mixture is incubated, and then the cuvette is moved to the wash station where the magnetic particles are washed. Acid Reagent is dispensed into the cuvette and then the cuvette is moved into the luminometer. The

addition of Base Reagent causes the chemiluminescent reaction to occur. The PMT measures the chemical light reaction that takes place.

There is one (1) main system operation key on the ACS:CENTAUR, the "**Sample Start button**". Pressing this key performs the following actions:

1. Homes the subsystems.
2. The system starts specimen sampling.
3. If the start button is pressed while the instrument is running, it stops sampling additional specimens; however it continues to process the specimens in the incubation ring.

Additional Equipment and Supplies

Reagent Water
Sample cups / tubes
Cuvettes
Sample tips
Reagent 1 (0.5% H_2O_2; 0.1N HNO_3)
Reagent 2 (less than 0.25N. Sodium Hydroxide and surfactant)
ACS:CENTAUR Cleaning Solution
ACS:CENTAUR primary and ancillary reagents.

VI. Calibration

The ADVIA Centaur T3 assay is traceable to an internal standard manufactured using U.S.P. (United States Pharmacopeia) material. Assigned values for calibrators are traceable to this standardization.

A two-point calibration must be performed at regular, assay specific intervals. Replicates for two calibrators of known value are processed. If the calibrators meet defined validity criteria, the system is adjusted. Refer to the Centaur Operating Procedures for calibration procedure.

A. Calibration Frequency:

Two-point calibration of the T3 assay is required:

1. every 28 days
2. when changing lot numbers of primary reagent packs
3. when replacing system components
4. when QC results are unacceptable

B. Calibration Material:

Use Calibrator A, High and Low Levels to perform two-point calibrations.

Actual concentrations may change with lot numbers.

1. Reconstitute each vial with 5.0 ml reagent grade water using a volumetric pipet
2. Let the calibrator stand for 15 – 20 minutes at room temperature.
3. Gently swirl and invert the vials until homogenous.
4. Each lot of calibrators contains a Calibrator Assigned Value card. Calibrator

values may be entered by using the barcode wand or the keyboard.

5. Affix the Low and High Calibrator barcode labels to the appropriate calibrator sample cups so that the system recognizes the sample as a calibrator.
6. Refer to the Centaur Operating Procedures for scheduling and running a calibration.

C. Stability / Storage:

1. Store at 2-8°C
2. Lyophilized calibrator stable until expiration date on the vial
3. Reconstituted calibrator stable 28 days.
4. On board stability 4 hours.

D. Precautions:

1. Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides.
2. Potential biohazard, human and/or other biological source material. Handle as if potentially infectious, according to established good laboratory practices.
3. Do not return any calibrators back into the vials after calibration because evaporation can occur, which may affect performance.
4. Dispose of any calibrator remaining in the sample cups after 4 hours.
5. Do not refill calibrator sample cups when the contents are depleted. If required, dispense fresh calibrators into new cups.

VII. Quality Control

A. QC product: BioRad Immunoassay Plus Levels 1, 2, and 3. BioRad, ECS Division, Anaheim,CA

B. Preparation/handling: Stored frozen. Allow thawing completely at either room temperature or at 2-8 °C. Stable for 30 days once thawed (unopened) at 2-8°C, 14 days thawed/opened.

C. Frequency: All three levels are run once per 24 hours, ideally one level per shift. (1st shift – Lev 1, 2nd shift – Lev 2, 3rd shift – Lev 3).

D. Acceptability Criteria – Acceptability of QC is determined by the lab internal QC policy. Corrective Acceptability of QC is determined by the lab internal QC policy and actions taken must be documented in the LIS system. No patient results may be released until QC results are acceptable.

VIII. Procedure

1. Computer order #16643
2. Prepare the sample container for each sample, ensuring that a barcode label is affixed.
3. Use the appropriately coded sample racks for the type of sample tube to be used:
   a. Position 1 – aliquot tube (blue screw cap)
   b. Position 2 – primary sample tube
   c. Position 3 – sample cup (Siemens)

4. Load each sample tube into a rack, ensuring that the barcode is visible through the slot in the rack.
5. Place the rack(s) in the entry queue.
6. Press 'START' **only** if the system is not currently 'In Process'. The analyzer will read the barcode label and run the appropriate tests via the LIS interface.
7. For the T3 assay, the system automatically performs the following steps:
   a. dispenses 50 ul of sample and 50µL of T3/T4/B12 ancillary reagent into a cuvette.
   b. dispenses 100 ul of Lite Reagent and 300 ul of Solid Phase and incubates for 7.5 minutes at 37°C.
   c. separates, aspirates, and washes the cuvettes with reagent water.
   d. dispenses 300 ul each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
   e. reports results (An inverse relationship exists between the amount of T3 present in the sample and the amount of relative light units, RLUs, detected by the system.

IX. Reporting Results

A. Calculations

The system automatically reports serum T3 results in ng/ml. For detailed information about how the system calculates results, refer to the system operating instructions.

B. Limitations of Procedure

Assay range (AMR):	0.1-5.0 ng/mL Results less than 0.1 should be reported out as "<0.1 ng/ml"
Automatic dilution:	Not applicable
Manual dilution	Values obtained >5.0 ng/mL should have a dilution performed using T3 Diluent. "Manual dilution" must be chosen and the dilution factor must be entered onto analyzer. **Maximum dilution factor: x 3** **Results above assay range with dilution shall be reported as ">15.0 ng/mL" in the LIS.**

C. Reference Interval: 0.60-1.81 ng/mL

D. Reporting

1. **Analytical measurement range (AMR):** 0.1-5.0 ng/ml

2. **Clinical reportable range (CRR):** 0.1-15.0 ng/ml. Report results less than 0.1 as “less than 0.1 ng/ml”.

X. Performance Specifications

1. Sensitivity: 0.1 ng/mL
2. Linearity: 0.1-5.0 ng/mL
3. Precision: MMCI inhouse studies:

Mean ng/mL	Within-run SD	Total SD
1.0351	0.0218	0.0471
1.9771	0.1020	0.1268
3.2341	0.0451	0.0769

XI. Procedural Notes/Problem-Solving Tips

1. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. 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.

Specimens that are . . .	***Have an insignificant effect on the assay up to . . .***
hemolyzed	250 mg/dL of hemoglobin
lipemic	1000 mg/dL of triglycerides
icteric	20 mg/dL of bilirubin

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

For additional information on performance characteristics including cross-reactivity and dilution recovery, see the product information in the ADVIA Centaur T3 product insert.

XI. References

1. Siemens Healthcare Diagnostics ADVIA Centaur T3 Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Centaur Reference Manual.
3. Siemens Healthcare Diagnostics ADVIA Centaur XP Operator's Guide.
4. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Approved Guideline, GP2-A5, 2006.
5. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry; Approved Guideline, EP7-A2, 2005.

Author: Theresa King MLS(ASCP)

Medical Director Approval: Robert Omiecki, PhD 2/4/2013

REVISION HISTORY (began tracking 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
0.1	Initial use. Transition from Siemens Vista platform.	T King	1/29/13

Reviewed

Coordinator	Date	Medical Director	Date
Theresa R King	2/4/2013	Robert Omiecki, PhD	2/4/2013