**Title:** T3

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| **Author:** | **Effective Date:**  *Note: The Effective Date is assigned after all approval signatures are obtained* | **Supersedes Procedure #** |
| Sue Baker |  | NEW |

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| **Revised By:** | **Date Revised** | **Effective (adopted) Date:**  *Note: The Effective Date is assigned after all approval signatures are obtained* |
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| **Approval Signature** | **Approval Date** |
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| Dr. Xiaolan Ou, Medical Director |  |
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|  |  |
| Mary Johnson, Chief Supervisor |  |
|  |  |
|  |  |
| Susan Baker, Assistant Supervisor |  |
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**REVISION HISTORY**

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| **Procedure #** | **Revision Date** | **Reason for Revision** |
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# TITLE:

1. Purpose

Triiodothyronine (T3) is the hormone principally responsible for the development of the effects of the thyroid hormones on the various target organs.

T3 (3,5,3’-triiodothyronine) is mainly formed extrrathyroidally, particularly in the liver, by enzymatic 5’-deiodination of T4. Accordingly, the T3 concentration in serum is more a reflection of the functional state of the peripheral tissue than the secretory performance of the thyroid gland.

A reduction in the conversion of T4 to T3 results in a decrease in the T3 concentration. It occurs under the influence of medicaments such as propranolol, glucocorticoids or amiodarone and in severe non-thyroidal illness (NTI), and is referred to as “low T3 syndrome”. As with T4, over 99% of T3 is bound to transport proteins. However, the affinity of T3 to them is around 10-fold lower.1,2,3,4

The determination of T3 is utilized in the diagnosis of T3-hyperthyroidis, the detection of early stages of hyperthyroidism and for indicating a diagnosis of thyrotoxicosis factitia.5,6,7

The Elecsys T3 assay employs a competitive test principle with polyclonal antibodies specifically directed against T3. Endogenous T3, released by the action of 8-anilino-1-napththalene sulfonic acid (ANS), competes with the added biotinylated T3-derivative for the binding sites on the antibodies labeled with the ruthenium complexa).

1. Tris(2,2’-bipyridyl(ruthenium(II)-complex(Ru(by)32+)
2. TEST PRINCIPLE

Competition principle. Total duration of assay: 18 minutes.

* 1st incubation: 30 uL of sample and a T3-specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by ANS.
* 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T3, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
* The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
* Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

1. SCOPE

Immunoassay for the in vitro quantitative determination of total triiodothyronine in human serum and plasma.

The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers

1. RESPONSIBILITIES

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| **Roles** | **Responsibilities** |
| Quality Assurance | Supports the process including provide leadership and/or assistance in support of the process.  Review and approval of procedure |
| Medical Director | Supports the development of the document.  Review and approval of the document. |
| Management | Review and approve the document.  Ensure that procedure is followed. |
| Laboratory Technical staff | Follows procedure. |

1. **ACRONYMS/DEFINITIONS**

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| URMC | University of Rochester Medical Center |
| VB12 | Vitamin B12 |
| SW | Strong West |
| RR | Ridgeland Road Laboratory |
| SMH | Strong Memorial Hospital |

1. **SPECIMENS**

For specimen collection and preparation only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Plasma: Na-heparin, Li-heparin, NH4+-heparin, K3- EDTA plasma, sodium citrate and sodium fluoride/potassium oxalate plasma.

Centrifuge samples containing precipitates before performing the assy. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators, and controls are at 20-25°C prior to measurement. Due to possible evaporation effects, samples, calibrators, and controls on the analyzers should be analyzed/measured within 2 hours.

Refer to SW.CP.GL.jad.0101 for sample stability.

1. **QUALITY CONTROL**

Analyze quality control materials as indicated on the Roche e411 analyzer set up form SW.CP.GL.frm.0102

1. **SPECIAL SAFETY PRECAUTIONS**

Exercise the normal precautions required for handling all laboratory reagents and biohazardous patient samples. Refer to Safety data sheets. Disposal of all waste material should be in accordance with local guidelines. Refer to Safety procedure SW.CP.GL.adm.0005

**VIII. MATERIALS**

**A. Equipment**

Roche cobas e 411 analyzer

Data Innovations Middleware

Bio-Rad Unity Real Time QC Application

**B. Supplies**

Roche Sample cups

Falcon tubes

Pipets

Pipet tips

**C. Reagents**

Elecsys T3 - (Ref # 11731360 122) – ready for use

Components:

The reagent pack is labeled as T3.

M – Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 – Anti-T3-Ab~Ru(bpy)32+ (gray cap), 1 bottle, 16 mL: Polyclonal anti-T3-antibody (sheep) labeled with ruthenium complex 75 ng/mL; ANS 0.8 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative

R2 – T3~biotin (black cap), 1 bottle, and 16 mL: Biotinylated T3 3 ng/mL; ANS 0.8 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Shelf life at 2-8°C see expiration date

After opening at 2-8°C 12 weeks

On-board 8 weeks

D. Calibrator

Calibrator T3 CalSet

Traceability: This method has been standardized against reference standards by weighing T3 into analyte-free human serum matrix.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

*Calibration frequency*: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

• after 8 weeks when using the same reagent lot

• after 7 days (when using the same reagent kit on the analyzer)

• as required: e.g. quality control findings outside the defined limits

1. **PROCEDURE**

Refer to general cobas e411 analyzer operating procedure SW.CP.GL.lab.0102

**IX. LIMITATIONS**

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. >5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL and samples from dialysis patients.

In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.

Therapy with amiodarone can lead to depressed T3 values.

Phenytoin, phenylbutazone, and salicylates cause release of T3 from the binding proteins, thus leading to a reduction in the total T3 hormone level at normal fT3 levels.8

Autoantibodies to thyroid hormones can interfere with the assay.

Binding protein anomalies seen with FDH (familial dysalbuminemic hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results.9

Pathological concentrations of binding proteins (TBG, albumin) can lead to total T3 values outside the normal range being found despite a euthyroid metabolic state (e.g. in NTI(b)-patients, pregnancy, use of oral contraceptives). In such cases a fT3 or fT4 determination is indicated.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

b) NTI = non thyroidal illness

**X. CALCULATIONS**

COBAS e411 analyzers automatically calculate the analyte concentration of each sample.

**XI. MEASURING RANGE AND DILUTIONS**

Refer to the Roche Range Chart for the measuring range and manual dilution guidelines (SW.CP.GL.jad.0104).

**XII. INTERPRETATION**

Refer to Reference Range guide for age appropriate reference ranges and critical value levels (SW.CP.GL.jad.0103).

**XII. RESULT REPORTING**

Results are generally reported via the DI Middleware-refer to procedure SW.CP.GL.lab.0103.

**XIII. TRAINING**

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| **Role** | **Training Needed** |
| Management | Read procedure |
| Employees | Read procedure |

**IVX. REFERENCES**

