Brown Clinic Northridge
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Watertown, SD

Brown Clinic
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Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

Free Thyroxine Flex® reagent cartridge (LOCI® Module)

INTENDED USE: The FT4L method is an *in vitro* diagnostic test for the quantitative measurement of Free Thyroxine in human serum and plasma on the Dimension® EXL™ integrated chemistry system with LOCI® Module. Measurements of free thyroxine are used in the diagnosis and monitoring of thyroid disease.

SUMMARY: Thyroxine is synthesized in the thyroid gland and once in the circulation, almost all thyroxine (99.97%) is protein bound. It is only the 0.03% unbound or "free thyroxine" that is capable of binding to cellular receptors resulting in a physiologic response.[1,2]

Free-T4 concentrations more closely parallel thyroid dysfunction in patients with either hypo- or hyperthyroidism than do the serum levels of total thyroxine.

PRINCIPLES OF PROCEDURE: The FT4L method is a homogeneous, sequential, chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-T4 mouse monoclonal antibody. The first bead reagent (Chemibeads) is coated with triiodothyronine (T3), a naturally occurring, weaker binding analog of T4, and contains chemiluminescent dye. The second bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. In a first step, sample is incubated with biotinylated antibody which allows T4 from the sample to saturate a fraction of the biotinylated antibody that is directly related to the free thyroxine (FT4) concentration. In a second step, T3 Chemibeads are added and form bead/biotinylated antibody immunocomplexes with the non-saturated fraction of the biotinylated antibody. Sensibeads are then added and bind to the biotin to form bead pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is an inverse function of the FT4 concentration in the sample.[3,4]

Reagents

Wells[a,b]	Form	Ingredient	Concentration[c]	Source
1 – 2	Liquid	Streptavidin Sensibeads	225 µg/mL	Recombinant *E. coli*
3 – 4	Liquid	T3 Chemibeads	200 µg/mL	
5 – 6	Liquid	FT4 Biotinylated antibody	50 ng/mL	Mouse monoclonal
7 – 8	Empty			

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Wells 1 – 6 contain buffers, stabilizers and preservatives.
c. Nominal value per well in a cartridge.

Risk and Safety:

H317
P280, P272, P302+P352, P333, P501
Warning!
May cause an allergic skin reaction.

Wear Protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains : 5-chloro-2-methyl-3 (2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.

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

Precautions: Used HM reaction vessels contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

For *in vitro* diagnostic use

Reagent Preparation: All reagents are liquid and ready to use.

Store at: 2 – 8 °C

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days.

Open Well Stability: 3 days for wells 1 – 6

SPECIMEN COLLECTION AND HANDLING: Recommended specimen types: Serum, lithium and sodium heparin, or EDTA plasma.

Samples and controls stabilized with sodium azide cannot be used.

Collect serum and plasma specimens using recommended procedures for collection of diagnostic blood specimens by venipuncture.[5]

Specimens must be free of particulate matter. To prevent the appearance of fibrin in serum samples, complete clot formation should take place before centrifugation. If clotting time is increased due to thrombolytic or anticoagulant therapy, the use of plasma specimens will allow for faster sample processing and reduce the risk of particulate matter.[7]

Follow the instructions provided with your specimen collection device for use and processing.[8]

The purpose of specimen storage information is to provide guidance to users; however, users may validate their own procedures for storing patient samples.

Specimen stability:

Processed samples may be refrigerated at 2 – 8 °C for 14 days if not tested within 24 hours.

For longer storage, serum samples may be frozen at -20 °C for three months.[6] Avoid repeated freezing and thawing.

PROCEDURE

Materials Provided

FT4L Flex® reagent cartridge, Cat. No. RF610

Materials Required But Not Provided

LOCI Thyroid Calibrator, Cat. No. RC610/RC610A
Reagent grade water (for use as Level 1 Calibrator)
HM reaction vessels, Cat. No. RXV1A
Quality Control Materials

Test Steps

Sampling, reagent delivery, mixing, and processing are automatically performed by the Dimension® EXL™ with LM System. For details of this processing, refer to your Dimension® EXL™ with LM Operator's Guide.

Test Conditions

Sample Volume (delivered to the HM reaction vessel)	10 µL
FT4 Biotinylated Antibody Reagent Volume	40 µL
T3 Chemibeads Reagent Volume	20 µL
Streptavidin Sensibeads Reagent Volume	60 µL
Temperature	37.0 °C
Reaction Time	12 minutes
Wavelength	Illumination 680 nm, Emission 612 nm
Type of Measurement	Chemiluminescence

CALIBRATION

Assay Range	0.1 – 8.0 ng/dL [1.3 – 103 pmol/L][d]
Calibration Material	LOCI Thyroid Calibrator Cat. No RC610/RC610A
Calibration Scheme	5 levels, n = 3
Units	ng/dL [pmol/L] (ng/dL x 12.87) = [pmol/L]
Typical Calibration Levels	Level 1* 0.0 ng/dL [0 pmol/L] *Level 1 is not included in the LOCI Thyroid Calibrator carton. Reagent grade water should be used as the level 1 calibrator for the FT4L method. Level 3: 0.8 ng/dL [10.3 pmol/L] Level 4: 1.6 ng/dL [25.7 pmol/L] Level 5: 4.0 ng/dL [51.5 pmol/L] Level 6: 8.4 ng/dL [108 pmol/L]
Calibration Frequency	Every 30 days for any one lot
A new calibration is required	• For each new lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures • When required by government regulations

d. Système International d'Unités [SI units] are in brackets.

Backup Process:

Refer to Brown Clinic Back-up Policy

CONTROLS:

At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

Results: The instrument calculates the concentration of free thyroxine in ng/dL [pmol/L] using the calculation scheme described in your Dimension® EXL™ with LM Operator's Guide.

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

Analytical Measurement Range (AMR): 0.1 – 8.0 ng/dL [1.3 – 103 pmol/L]

This is the range of analyte values that can be measured directly from the specimen without any dilution or pretreatment that is not part of the analytical process and is equivalent to the assay range.

- Samples with results in excess of 8.0 ng/dL [103 pmol/L] should be reported as greater than 8.0 ng/dL [103 pmol/L]. **Samples should NOT be diluted.**
- Samples with results less than 0.1 ng/dL [1.3 pmol/L] should be reported as "less than 0.1 ng/dL [1.3 pmol/L]".

Limitations of Procedure

Patient samples may contain heterophilic antibodies that could react in immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.[9,10] Thyroid autoantibodies in human serum may interfere and cause falsely elevated FT4L results.

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in FT4L results. Refer to your Dimension® EXL™ with LM Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed according to your laboratory's procedure manual and not reported.

United States Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the laws of the State in which he practices, to use or order the use of the device.

Expected Values:

Adult: 0.76 – 1.46 ng/dL [9.8 – 18.8 pmol/L]

Pediatric:

Infants (1-23 months)	0.93-1.45 ng/dL [12.0-18.7 pmol/L]
Children (2-12 years)	0.82-1.40 ng/dL [10.6-18.0 pmol/L]
Adolescents (13-20 years)	0.78-1.34 ng/dL [10.0-17.3 pmol/L]

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: FT4L Flex® reagent cartridge insert sheet PN 741610.001 Issue Date 2015-04-21

Origination Date: 8-11-12
Date of Implementation: 9-1-12
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-11-12_____

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
5/12/15	Heather Hall	Updated Format of Specimen Stability, Removed Statistical items and referenced package insert
7/21/15	Heather Hall	Updated precautions and pediatric expected values
09-24-2015	Sam Legg	Updated Risk and Safety and added comment per United States Federal law
2/21/17	Lori Murray	backup method clarification
10/18/17	Heather Hall	Modified backup method