TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	11/20/2015
Department:	Core	**Due Date:**	12/14/2015
		Implementation:	**12/15/2015**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Thyroid Stimulating Hormone by Dimension Vista® System	**SGAH.C84, WAH.C80 v2**
Free Thyroxine by Dimension Vista® System	**SGAH.C85, WAH.C81 v2**
Gamma Glutamyl Transferase by Dimension Vista® System	**SGAH.C103, WAH.C92 v2**
Human Chorionic Gonadotropin, Quantitative by Dimension Vista® System	**SGAH.C107, WAH.C103 v3**

Description of change(s):

Most changes are minor and the following apply to all of them

Section	Reason
3.2	Specify anticoagulant
4.2	Add hazard information
5.2	Delete opened off board stability
6.4, 6.6	Replace LIS with Unity Real Time

Free Thyroxine only

Section	Reason
8	Update FT4 reagent volume
14.3	Add interfering substances

A copy of the Free Thyroxine SOP is attached with changes highlighted

These revised SOPs will be implemented on December 15 , 2015

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training (version 2)

Technical SOP

Title	**Free Thyroxine by Dimension Vista® System**	
Prepared by	Ashkan Chini	Date: 6/25/2012
Owner	Robert SanLuis	Date: 10/21/2013

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Form revised 2/02/2007

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Free Thyroxine	Dimension Vista® System	FT4

Synonyms/Abbreviations
Free T4

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The FT4 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 T4 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. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum	
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)	
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL	
Transport Container and Temperature	Collection container or Plastic vial at room temperature	
Stability & Storage Requirements	Room Temperature:	24 hours
	Refrigerated:	14 days
	Frozen:	3 months

Form revised 2/02/2007

Criteria	
	Instrument on board aliquot stability 2 hours
Timing Considerations	Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST to clot completely prior to centrifugation.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Free Thyroxine	Siemens, Flex® reagent cartridge, Cat. No. K6410

4.2 Reagent Preparation and Storage

NOTES: Each container must be labeled with (1) substance name, (2) lot number, (3) expiration date, (4) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration. Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

May cause an allergic skin reaction.
Wear protective clothing, gloves and eye/face protection.

Reagent	**Free Thyroxine**
Container	Reagent cartridge

Storage	Store at 2-8° C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Once wells 1 - 12 have been entered by the instrument, they are stable for 7 days.
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
LOCI 1 CAL	Siemens Dimension Vista®, Cat. No. KC660

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

Calibrator	LOCI 1 CAL
Preparation	Thaw and equilibrate at room temperature (22 – 28° C) for at least 30 minutes. Mix the contents of the box by inverting gently 10 times.
Storage/Stability	• Store at -25 to -15° C • **Unopened calibrator** is stable until expiration date stamped on the box. • **Opened Calibrator:** once the stopper of the vial is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista System.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	LOCI 1 CAL
Assay Range	0.10 – 8.00 ng/dL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/dL

Frequency	• Every new reagent cartridge lot. • Every 30 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	5 levels, n = 3

5.4 Calibration Procedure

Auto Calibration:

1. Place the required calibrator vials in a carrier. Make sure the barcode labels are entirely visible through the slots.
2. Place the carrier in the loading area.
3. Position the carrier with the labels facing away from the user.
4. Press the **Load** button.
5. Automatic calibration requires that calibrators be on the instrument. As the time for processing approaches, the instrument reviews onboard inventory for the appropriate calibrators.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.
3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

5.5 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Immunoassay Plus Control Levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 361, 362 and 363

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation. A barcode label is produced and placed on the vial.

Control	Liquichek Immunoassay Plus Controls, Level 1, 2 and 3
Preparation	Allow to stand at room temperature (18-25°C) until completely thawed. Before sampling, gently swirl the vial several times gently to ensure homogeneity. Promptly replace the stopper and return to 2-8°C storage after each use.
Storage/Stability	• This product will be stable until the expiration date when stored unopened at -20 to -70° C. • **Thawed and unopened:** all analytes will be stable for 30 days. • **Thawed and opened:** all analytes will be stable for 14 days when stored tightly capped at 2-8° C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Dimension Vista® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Vista® Quick Reference Guide.

6.4 Tolerance Limits

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC

Step	Action
	Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Review Patient Data

Technologist must review each result with error messages. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.7 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples.

Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.

- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Dimension Vista® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

- Aliquot Plates
- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

FT4 Flex® reagent cartridge Cat. No. K6410 is required to perform this test.

Free Thyroxine is performed on the Dimension Vista® System after the method is calibrated (see Reference Material in Calibration section) and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

8.1	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
4.	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions	
Sample Volume:	10 µL
FT4 Biotinylated Antibody Reagent Volume:	40 µL
T3 Chemibeads Reagent Volume:	20 µL
Streptavidin Sensibeads Reagent Volume:	60 µL
Reaction Time:	10 minutes
Test Temperature:	37° C
Wavelength:	680 & 612 nm
Type of measurement:	Chemiluminescence

9. CALCULATIONS

The instrument automatically calculates the concentration of Free Thyroxine in ng/dL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results up to two decimal points.

10.3 Units of Measure

ng/dL

10.4 Clinically Reportable Range (CRR)

0.10 – 8.00 ng/dL

10.5 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 0.10 ng/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.10 ng/dL
> 8.00 ng/dL	Repeat the assay. If replicates agree within the TEa, report as: "> 8.00 ng/mL-REP". Do not dilute Free T4

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

Free T4	**Male**	**Female**
Adult (>18 years):	0.76 – 1.46 ng/dL	0.76 – 1.46 ng/dL
Pediatric:		
0 - 3 days	0.97 - 1.87	0.93 – 1.44
4 - 30 days	0.78 - 1.52	0.81 – 1.44
1 - 11 months	0.89 - 1.48	0.93 – 1.40
1 - 5 years	0.97 – 1.25	1.01 – 1.32
6 - 10 years	0.93 – 1.32	0.93 – 1.25
11 - 15 years	0.97 – 1.25	0.89 – 1.20
16 - 18 years	0.97 – 1.25	0.93 - 1.25

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Thyroxine is synthesized in the thyroid gland and once in the circulation, almost all thyroxine is protein bound. It is only the few unbound or "free thyroxine" that is capable of binding to cellular receptors resulting in a physiologic response.
FT4 concentrations more closely parallel thyroid dysfunction in patients with either hypo- or hyperthyroidism than do the serum levels of total thyroxine.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension Vista Operator's Guide.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following Free Thyroxine concentrations are:

FT4 Concentration	Acceptable S.D. Maximum
0.90 ng/dL	0.10 ng/dL
2.00 ng/dL	0.20 ng/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.10 – 8.00 ng/dL

14.2 Precision

Material	Mean ng/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Liquichek Immuno. Control			
Level 1	0.75	0.01 (1.96)	0.02 (2.07)
Level 2	1.80	0.03 (1.74)	0.05 (2.66)
Level 3	5.28	0.09 (1.44)	0.13 (2.11)

14.3 Interfering Substances

- Carbamazepine, Diclofenac, Furosemide, Ibuprofen, Linoleic Acid, Mefenamic acid, N-acetylcysteine, Oleic acid, Phenylbutazone, Phenytoin and Salicylic acid will all falsely elevate Free Thyroxine result.
- Patient samples may contain heterophilic antibodies that could react in immunoassays to give falsely elevated or depressed result. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.
- Thyroid autoantibodies in human serum may interfere and cause falsely elevated FT4 results.

HIL Interference:
The FT4 method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered “interference”.

Substance tested	Substance Concentration	FT4 ng/dL	Bias %
Hemoglobin (hemolysate)	750 mg/dL	1.10	<10
Bilirubin (unconjugated)	30 mg/dL	1.10	<10
Bilirubin (conjugated)	26 mg/dL	1.10	<10
Lipemia Intralipid®	3000 mg/dL	1.10	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. QC Schedule for Siemens Dimension Vista®
8. Laboratory Safety Manual
9. Material Safety Data Sheets (MSDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Quest Diagnostics Records Management Procedure
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
14. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert FT4 Flex® Reagent Cartridge K6410

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144.
2. Package Insert, FT4 Flex® Reagent Cartridge K6410, Siemens Healthcare Diagnostics Inc., 03/20/2015.
3. Package Insert, LOCI 1 CAL, Siemens Healthcare Diagnostics Inc., 03/2015.
4. Package Insert, Liquichek Immunoassay Plus Control, Bio-Rad Laboratories, 11/2014.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	10/21/13		Update owner	L Barrett	R SanLuis
000	10/21/13	11.1	Change adult range	L Barrett	R SanLuis
000	10/21/13	16	Update titles	L Barrett	R SanLuis
000	10/21/13	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
1	10/30/15	3.2	Specify anticoagulant	L Barrett	R SanLuis
1	10/30/15	4.2	Add hazard information	A Chini	R SanLuis
1	10/30/15	5.2	Delete opened off board stability	A Chini	R SanLuis
1	10/30/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
1	10/30/15	8	Update FT4 reagent volume	A Chini	R SanLuis
1	10/30/15	14.3	Add interfering substances	A Chini	R SanLuis
1	10/30/15	17	Update Package Insert dates	A Chini	R SanLuis

19. ADDENDA

None