TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	5/6/2015
Department:	Core	**Due Date:**	6/2/2015
		Implementation:	**6/3/2015**

DESCRIPTION OF PROCEDURE

Name of procedure:

Human Chorionic Gonadotropin, Quantitative by Dimension® Xpand Chemistry Analyzer GEC.C27 v2

Description of change(s):

Section	Reason
3.2	Specify lithium heparin anticoagulant
1,7.1	Add analyzer name
6.4, 6.6	Replace LIS with Unity Real Time
6.7	Add use of TEA for lot to lot runs
8.2	Remove Lynx, specify Xpand process
10.5	Revise manual dilution process, remove code QNSR

This revised SOP will be implemented on June 3, 2015

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

Approved draft for training

Technical SOP

Title	**Human Chorionic Gonadotropin, Quantitative by Dimension® Xpand Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 4/12/2011
Owner	Robert SanLuis ~~Jean Buss~~ Date: 4/13/2015

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**

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TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Human Chorionic Gonadotropin, Quantitative	Dimension® Xpand Chemistry Analyzer	HCGQ

Synonyms/Abbreviations
Pregnancy test, Quant/ Quant hCG

Department
Chemistry

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2. ANALYTICAL PRINCIPLE

The HCG method is a two-step enzyme immunoassay based on the "sandwich" principle. Sample is incubated with chromium dioxide particles, coated with monoclonal antibodies specific for the hCG alpha subunit, to form a particle/hCG complex. Particles are separated magnetically and the supernatant is removed. During a second step the particle/hCG complex is incubated with conjugate reagent (ß-galactosidase labeled monoclonal antibodies specific for the hCG beta subunit) to form a particle/hCG/conjugate sandwich. Unbound conjugate is removed by magnetic separation and washing. The sandwich bound ß-galactosidase is combined with the chromogenic substrate chlorophenol red-ß-d-galactopyranoside (CPRG) and catalyzes the hydrolysis of the substrate to chlorophenol red (CPR). The concentration of hCG in the patient sample is directly proportional to the rate of color change due to formation of CPR measured at 577 and 700 nm.

hCG + CrO_2-Ab → CrO_2-Ab-hCG + CrO_2-Ab

CrO2-Ab-hCG + CrO2-Ab + F(ab')2-ß-galactosidase → CrO2-Ab-hCG-F(ab')2-ß-gal + CrO2-Ab + F(ab')2-ß- galactosidase

Wash

CrO2-Ab-hCG-F(ab')2-ß-gal + CrO2-Ab + F(ab')2-ß-galactosidase → CrO2-Ab-hCG-F(ab')2-ß-gal + CrO2- Ab (transferred to cuvette)

CrO_2-Ab-hCG-F(ab')2-ß-gal

CPRG (non-absorbing at 577 nm) ----------> CPR (absorbs at 577 nm)

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	None
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection tube or plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: (2-8°C) 48 hours
	Frozen: (-20°C or colder) 2 months
Timing Considerations	N/A
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 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
Human Chorionic Gonadotropin	Siemens, Flex® reagent cartridge, Cat. No. RF430
Sample Diluent	Dimension® clinical chemistry system, REF791092901

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

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.

Irritant. Contains mixture of 5-chloro-2-mtheyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). **May cause sensitization by skin contact.**

Reagent	**Human Chorionic Gonadotropin**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. • Once wells 1 – 2 have been entered by the instrument, they are stable for 5 days. • Once wells 4 – 6 have been entered by the instrument, they are stable for 3 days. • Once wells 3, 7 and 8 have been entered by the instrument, they are stable for 10 days.
Preparation	Hydrating, diluting and mixing are automatically performed by the instrument.

Reagent	**Sample Diluent**
Container	Manufacturer supplied vial
Storage	Store at 2-8° C
Stability	Sample diluent, opened or unopened product, is stable until the expiration date stamped on the vial.
Preparation	Ready for use. No preparation is required.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
HCG Calibrator	Siemens Dimension®, Cat. No. RC430

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) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	Human Chorionic Gonadotropin Calibrator
Preparation	Volumetrically add 2.00 ± 0.02 mL of Millipore® or reagent grade water to each vial. Replace stopper, invert gently 10 times and swirl gently for 10 seconds. Let stand or gently mix for 15 to 20 minutes until the cake is completely dissolved. Use immediately or refrigerate at 2–8°C for future use. Swirl gently before use.
Storage/Stability	• Store at 2-8°C. • The unopened reagents are stable until the expiration date printed on the label. • Opened product is stable for 24 hours when stoppered and stored at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Human Chorionic Gonadotropin Calibrator
Assay Range	1 – 1000 mIU/mL
Suggested calibration level	See Reagent Package Insert for lot specific assigned values in mIU/mL
Frequency	• Every new reagent cartridge lot. • Every 2 months for any one lot. • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Five levels.
Assigned Coefficients	C_0 -6900 C_1 17500 C_2 -0.6 C_3 5000 C_4 0.5

5.4 Calibration Procedure

1. From Operating Menu press F5:Process Control press F1: Calibration Enter Password press F2: SETUP and RUN
2. Select the test method to be calibrated - if lot number is incorrect Press F1: Other Lot
3. Enter all information on screen
4. Press F8: QC yes/no to change to yes
5. Press F4: Assign cups If additional methods need to be calibrated, select the method.
6. Press F7: Load/run
7. Load cups into assigned position
8. Press F4: RUN

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 & 3	Bio-Rad Laboratories Cat. No. 361, 362 & 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.

Control	Liquichek Immunoassay Plus Control, Levels 1, 2 & 3
Preparation	Allow the frozen control to stand at room temperature (18-25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Open controls are stable for 14 days at 2-8°C. Unopened controls are stable until the expiration date at -20 to -70°C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing.

Refer to the Dimension® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension® 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 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.

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Step	Action
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 Xpand® 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 Xpand® 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

- Plastic serum tubes and serum cups
- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips
- Reaction Vessels, Cat. No. RXV1A
- Chemistry Wash, Cat. No. RD701
- Probe Cleaner, Cat. No. RD702
- Sample Probe Cleaner, Cat. No. RD703
- IMT Probe Cleaner, Cat. No. RD704

8. PROCEDURE

HCG Flex® reagent cartridge Cat. No. RF430 is required to perform this test.

Human Chorionic Gonadotropin is performed on the Dimension® clinical chemistry 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	Instrument Set-Up Protocol
1.	For instrument set up and operation: Refer to Startup and Maintenance, Siemens Dimension® Xpand procedure.
2.	Check reagent inventory
3.	Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® Xpand system. For details of the automated parameters, see below under “Test conditions.”

8.2	Specimen/Reagent Preparation
1.	Centrifuge the specimens.
2.	Specimens are placed in Dimension® Xpand segments for analysis by the instrument. Refer to the Sample Processing, Siemens Dimension® Xpand procedure. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus 50 µL of dead volume. Precise container filling is not required.

8.3	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension® Xpand QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension® Xpand Operators 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® Xpand 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	
Reaction Vessel	
Step one	
Sample Size:	40 µL
Antibody-CrO_2:	40 µL
Incubation Temp:	42° C
Incubation Period:	3.5 minutes
Chrome Separation:	1.2 minutes
Step two	
HCG Ab-ß-galactosidase:	110 µL
Incubation Period:	3.7 minutes
Wash Steps:	4.1 minutes (2 washes)
Cuvette	
Transfer Volume:	50 µL
Substrate Reagent Volume (CPRG):	175 µL
Diluent Volume:	165 µL
Reaction Time:	1.5 minutes
Wavelength:	577 and 700 nm
Type of Measurement:	Bichromatic rate

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of HCG in mIU/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

mIU/mL

10.4 Clinically Reportable Range (CRR)

1 – 1000,000 mIU/mL

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…
< 1 mIU/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 1 mIU/mL
≥1,000 mIU/mL	**On Board Automated Dilution:** Results ≥1,000 mIU/mL will automatically have repeat testing performed into the instrument using dilution factor of 200. No multiplication is necessary.
>200,000 mIU/mL	**Manual Dilution:** Using the primary tube, make a 1:5 dilution. Program the sample manually on the instrument and change the "Dilution Factor" to 5. No multiplication is necessary; the instrument will automatically perform all calculations. **Diluent**: Sample Diluent

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>1,000,000 mIU/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "<1,000,000 mIU/mL-REP". Bring to the attention of your supervisor prior to releasing result.

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

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Non- Pregnant Female	Male
All	0-6 mIU/mL	0-2 mIU/mL

HCG levels with Gestational Age

Gestational Age	hCG mIU/mL
0.2-1 week	5-50
1-2 weeks	50-500
2-3 weeks	100-5,000
3-4 weeks	500-10,000
4-5 weeks	1,000-50,000
5-6 weeks	10,000-100,000
6-8 weeks	15,000-200,000
2-3 months	10,000-100,000

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Human chorionic gonadotropin (hCG) is a sialoglycoprotein hormone produced by the placenta soon after implantation of the fertilized ovum into the uterine wall. The presence of hCG in serum shortly after conception, followed by its rapid rise in concentration, makes it an excellent marker for confirmation and monitoring of pregnancy. Physiologically, hCG appears to maintain the corpus luteum and support the endometrium. Serum hCG levels increase to a peak concentration during the first trimester, then decrease and plateau during the remainder of pregnancy. hCG circulates as the intact molecule in the serum of normal women who have an uncomplicated pregnancy.

The concentration of HCG rises rapidly during the first weeks of pregnancy, approximately doubling every two days. Low level HCG values > 25 mIU/mL [IU/L] may be indicative of

early pregnancy, but these results should always be evaluated in the context of the clinical situation: date of last menstrual period, pelvic examination and other clinical findings.

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 Operator's Guide.

A system malfunction may exist if the following 5-test precision is observed:

Concentration	S.D.
25 mIU/mL	> 2.5 mIU/mL
150 mIU/mL	> 11.0 mIU/mL
500 mIU/mL	>37.0 mIU/mL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

1 – 1000 mIU/mL

14.2 Precision

Material	Mean mIU/mL	Standard Deviation (%CV)	
		Within-run	Total
Serum Pool			
Level 1	0.93	0.57	0.83
Level 2	103.48	1.56	2.98

14.3 Interfering Substances

No clinically significant interference was observed from icterus (bilirubin 60 mg/dL, hemolysis (hemoglobin 1000 mg/dL) or Lipemia (triglyceride 3000 mg/dL).

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 Xpand® Clinical Chemistry System Operator's Manual
2. Calibration / Verification Siemens Dimension® Xpand procedure
3. Dimension Xpand® Cal Accept Guidelines
4. Dimension Xpand® Calibration summary
5. Sample Processing, Siemens Dimension® Xpand procedure
6. Start up and Maintenance, Siemens Dimension® Xpand procedure
7. Laboratory Quality Control Program
8. QC Schedule for Siemens Dimension Xpand®
9. Laboratory Safety Manual
10. Material Safety Data Sheets (MSDS)
11. Siemens Dimension Xpand® Limits Chart (AG.F143)
12. Quest Diagnostics Records Management Procedure
13. Dimension Xpand® System Error Messages Chart
14. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
15. Hemolysis, Icteria and Lipemia Interference (Lab policy)
16. Repeat Testing Requirements (Lab policy)
17. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
18. Current package insert HCG Flex® Reagent Cartridge RF430

17. REFERENCES

1. Package Insert, HCG Flex® Reagent Cartridge RF430, Siemens Healthcare Diagnostics Inc., 11/27/2012.
2. Package insert, Human Chorionic Gonadotropin Calibrator RC430, Siemens Healthcare Diagnostics Inc., 06/2012.
3. Package insert, Liquichek Immunoassay Plus Control, Bio-Rad Laboratories, 11/2014.
4. Package insert, Sample diluent REF791092901, 01/2010.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C076.001		
000	5/9/2011		Update owner	L Barrett	J Buss
000	5/9/2011	10.5	Corrected diluent for manual dilution	A Chini	J Buss
001	4/13/15		Update owner	L Barrett	R SanLuis
001	4/13/15	3.2	Specify lithium heparin anticoagulant	L Barrett	R SanLuis
001	4/13/15	1,7.1	Add analyzer name	L Barrett	R SanLuis
001	4/13/15	6.4, 6.6	Replace LIS with Unity Real Time	A Chini	R SanLuis
001	4/13/15	6.7	Add use of TEA for lot to lot runs	L Barrett	R SanLuis
001	4/13/15	8.2	Remove Lynx, specify Xpand process	L Barrett	R SanLuis
001	4/13/15	10.5	Revise manual dilution process, remove code QNSR	A Chini L Barrett	R SanLuis
001	4/13/15	15	Update to standard wording	L Barrett	R SanLuis
001	4/13/15	16	Update SOP titles	L Barrett	R SanLuis
001	4/13/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis

19. ADDENDA

None