Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Human Chorionic Gonadotropin

PURPOSE:
The HCG method for the Dimension® clinical chemistry system with the heterogeneous immunoassay module is intended to quantitatively measure intact human chorionic gonadotropin in **serum** and **plasma** for the early detection of pregnancy.

Human choronic gonadotropin (hCG) is a sialoglycoprotein hormone produced by the placenta soon after implantation of the fertilized ovum into the uterine wall. LHCG Flex ® reagent cartridge (Cat. No. RF530) contains fewer tests per Flex ® than the standard HCG Flex ® reagent cartridge (RF430). 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.

PRINCIPLE:
The LHCG 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 (B-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 B-galactosidase is combined with the chromogenic substrate chlorophenol red-B-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 577/700 nm.

$$hCG + CrO_2\text{-}Ab \longrightarrow CrO_2\text{-}Ab\text{-}hCG + CrO_2\text{-}Ab$$

$$CrO_2\text{-}Ab\text{-}hCG + F(ab')_2\text{-}\beta\text{-galactosidase} \longrightarrow CrO_2\text{-}Ab\text{-}hCG\text{-}F(ab')_2\text{-}\beta\text{-gal} + F(ab')_2\text{-B-galactosidase}$$

$$CrO_2\text{-}Ab\text{-}hCG\text{-}F(ab')_2\text{-}\beta\text{-gal} + \xrightarrow{\text{Wash}} CrO_2\text{-}Ab\text{-}hCG\text{-}F(ab')_2\text{-}\beta\text{-gal} +$$

$F(ab')_2$-β-galactosidase CrO_2-Ab (transferred to cuvette)

CPRG CrO_2-Ab-hCG-$F(ab')_2$-β-gal CPR
(Non-absorbing at 577nm) ⟶ (absorbs at 577nm)

Reagents[a]

Wells[b]	Form	Ingredient	Concentration[c]	Source
1, 2	Liquid	hCG Ab-ß-galactosidase	d	Mouse, monoclonal
3	Tablet[d]	Antibody-CrO_2	0.75 mg/mL[d]	Mouse, monoclonal
4, 5, 6	Tablet[d]	CPRG	23.6 mM	
7	Liquid	Substrate Diluent Buffer	175 mM	
8	Liquid	Chrome Diluent Buffer	105 mM	

a. Each Flex® reagent cartridge contains 15 tests.
b. Wells are numbered consecutively from the wide end of the cartridge.
c. Nominal value in hydrated cartridge.
d. Antibody titer and conjugate activity may vary from lot to lot.
e. Tablets contain excipients, buffers, and stabilizers.

Risk and Safety:
H317
P280, P272, P302 + P352, P333 + P313, 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 cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

For *in vitro* diagnostic use

Reagent Preparation: Hydrating, diluting and mixing are automatically performed by the instrument.

Reagent Storage Instructions: Store at 2 - 8°C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. Once wells 1 and 2

have been entered by the instrument, they are stable for 5 days. Once wells 4, 5 and 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.

SPECIMEN

Specimen collection:

Serum or plasma can be collected and stored by normal procedures for collection of diagnostic blood specimens by venipuncture.

Follow instructions provided with your specimen collection device for use and processing.

Specimens should 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.

Frozen plasma samples with insufficient anticoagulants potentially can cause clumping of chrome particles and should not be used.

EDTA, Lithium heparin and sodium heparin anticoagulants do not interfere with the LHCG method.

Blood collected in the presence of oxalate potentially can cause clumping of the chrome particles and should not be used.

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

Specimen:

Patient preparation: no patient preparation required

Specimen type: serum or heparinized plasma

Stability: Separated Samples

Room Temp: 24 hours

Refrigerated at 2-8°C: up to 48 hours

Frozen at -20°C: up to 2 months, Mix thoroughly after thawing. Avoid repeated freezing and thawing.

Procedure

Materials Needed

LHCG Flex® reagent cartridge, Cat. No. RF530

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

Sample Diluent, Cat. No. 791092901

HCG Calibrator, Cat. No. RC430

Quality control materials

Test Steps Sampling, reagent delivery, mixing, separation, processing and printing of results are automatically performed by the Dimension® system with the heterogeneous immunoassay module. For details of this processing, refer to your Dimension® system manual.

Test Conditions

Reaction vessel
Step one

- Sample size: 40 µL
- Antibody-CrO_2: 40 µL
- Temperature: 37.0° 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: 175 µL
- Diluent Volume: 165 µL
- Wavelength: 577 and 700 nm
- Type of measurement: Bichromatic Rate

Calibration The general calibration procedure is described in your Dimension® system manual (also see Appendix B). The following information should be considered when calibrating the HCG method:

- Assay Range 1 - 1000 mIU/mL [IU/L]
- Reference Material HCG Calibrator Cat. No. RC430
- Suggested Calibration Levels 0, 25, 150, 500, 1000 mIU/mL [IU/L]
- Units mIU/mL [IU/L]
- Calibration Scheme, Replicates: 3@ Level 1, 2@ Level 2, 3, 4, 3@ Level 5

• Calibration Frequency:	Every new reagent cartridge lot			
	Every 2 months for any one lot			
	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			
• Assigned Coefficients:	C_0	-6900	C_3	5000
	C_1	17500	C_4	0.5
	C_2	-0.6		

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 automatically calculates and prints the concentration of HCG in mIU/mL [IU/L].

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

Limitations of Procedure

Analytical Measurement Range (AMR): 1-1000 mIU/mL [IU/L]

Autodilution (AD):	Autodilute assay range 200,000 mIU/mL [IU/L]
	Autodilute Volume 2 µL.
Manual dilution:	Results > 200,000 mIU/mL [IU/L] should be repeated after diluting the sample.
	Prepare a 1:2 dilution of the sample with Sample Diluten.
	Enter dilution factor of "2" in the Enter Data Screen.
	Instrument will automatically perform a 1:200 dilution on the manually diluted sample.
	The final HCG result calculated by the instrument.
Results:	<1 mIU/mL [IU/L] should be reported as "less than 1 mIU/mL [IU/L]" instead of the numerical value.

The HCG method is a two-step assay that removes excess hCG prior to the addition of conjugate reagent, thereby minimizing "hook effect."[5] The Dimension® HCG method shows no hook effect up to at least 1,000,000 mIU/mL [IU/L] hCG.

Patient samples may contain human heterophilic antibodies that could react with immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophilic antibodies.[6] 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.

Elevated hCG levels have also been associated with trophoblastic disease and nontrophoblastic neoplasms. The possibility of having these diseases should be considered before a diagnosis of pregnancy is made.[3,7] This test is not intended for use as a surrogate marker for aiding in the diagnosis of monitoring the treatment of cancer patients.

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® system manual.

Expected Values

		Range	
Population	Age	mIU/mL [IU/L]	n
Non pregnant female	22 - 87	0 - 6	104
Male Adult	19 - 83	0 - 2	95

The expected values were calculated non-parametrically and represent the central 95% of the population. Each laboratory should establish its own expected values for hCG as performed on the Dimension® system.

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, (see Limitations of Procedure section).[8] When borderline results are encountered, patient samples should be redrawn 48 hours later.[9,10]

The concentration of HCG rises rapidly during pregnancy. A maximum level of 5000 to 200,000 mIU/mL is reached at 10-12 weeks, followed by a slow decline to levels of 1000 to 50,000 mIU/mL during the third trimester.[1,3,11,12] A reduced or declining HCG level may indicate an abnormal pregnancy and additional follow-up testing and clinical evaluation should occur. Throughout the entire pregnancy, HCG levels vary with different gestational ages. See table below.

HCG levels with Gestational Age[11]

Gestational Age	hCG mIU/mL [IU/L]
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

Reporting:
report format: IU/ml

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: HCG Flex® reagent cartridge insert sheet PN 755430.001 Issue Date 2015-02-18

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Written By: _Lori Murray, MT(ASCP)_____ Date: _7-13-10_____

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

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New Format
11-10	Lori Murray	Chg control material from Biorad Liq Multiqual to HCG Control Set
5/22/15	Heather Hall	Updated information to Package insert dated 11/27/2012
01/19/16	Sam Legg	Updated to include Risk and Safety per Package insert dated 02-18-2015
2-28-17	Lori Murray	added backup method
10/18/17	Heather Hall	Modified backup process