MAYO CLINIC LABORATORIES

Test Definition: IEHCG

Interference Evaluation Heterophile, Beta-Human Chorionic Gonadotropin, Serum

Overview

Useful For

Evaluating suspected interference from heterophile antibodies causing a falsely elevated human chorionic gonadotropin result

This test is **not to be used** for pregnancy testing.

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
HCGII	HCG, Interference Interpretation	No	Yes
HCGQN	Beta-HCG, Quantitative, S	Yes, (order BHCG)	Yes
HCGAM	HCG, Alternative Method, S	No	Yes

Testing Algorithm

This heterophile antibody evaluation consists of pretreatment with commercial heterophile antibody blocking reagents, testing on an alternate platform, and serial dilution of the sample.

Highlights

The specimen will be evaluated for potential heterophile antibody interference in the total beta- human chorionic gonadotropin (hCG) immunoassay.

The presence of heterophile interference is not suspected when the alternate platform evaluation, dilution, and heterophile blocking tube pretreatment do not substantially alter measured beta-hCG concentrations.

Method Name

HCGQN: Electrochemiluminescent Immunoassay
HCGAM: Immunoenzymatic Assay

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

If "HCG Total OB" or pregnancy is indicated, order THCG / Human Chorionic Gonadotropin (hCG), Quantitative, Pregnancy, Serum.

Specimen Required

Patient Preparation: For 12 hours before specimen collection, do not take multivitamins or dietary supplements containing biotin (vitamin B7), which is commonly found in hair, skin, and nail supplements and multivitamins.
Supplies: Sarstedt Aliquot Tube 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 2.5 mL
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume

1.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	7 days	
	Frozen	90 days	
	Ambient	7 days	

Clinical & Interpretive

Clinical Information

Due to exposure to animal antigens, some patients have developed antibodies that interfere with immunoassay testing. These heterophilic antibodies can bind to animal antibodies used in immunoassays. It has been found that a significant percentage of certain sandwich immunoassay results are false-positive results caused by heterophilic antibody interference. The most frequently reported assay interference effect of heterophilic antibodies is a false-positive assay result. False-negative assay results have also been reported in the literature. Manufacturers add blocking agents to their reagents, but occasional patient samples containing heterophile antibodies are incompletely blocked. Subsequent reporting of erroneous results can have adverse effects on patient management, especially with tumor marker assays.

Among immunometric assays, human chorionic gonadotropin (hCG) assays have been found uniquely susceptible to heterophile antibody interference, resulting in occasional false-positive results. The current assay has proven robust in this respect, but rare interferences still occur. Typically, the observed false-positive elevations are modest, ranging from just above the reference range to levels of 50 to 60 IU/L. If such results are seen and are discordant with the clinical picture or other biochemical or imaging tests, then the laboratory should be alerted. After additional blocking treatment, repeat analysis of the specimen in question may resolve the issue. Dilution of the specimen prior to assay performance often yields unexpected nonlinear results in the presence of interfering substances, such as heterophile antibodies. Heterophile blocking tube treatment is used for troubleshooting samples that yield results that are either nonlinear or do not match the clinical picture of the patient and are suspected of containing heterophile antibodies. Finally, assessment of an analyte, such as hCG with an alternative assay will often lead to apparent discrepant results in the presence of a heterophile antibody, as heterophile antibodies often interact differently with alternative assay antibodies.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone (molecular weight [MW] approximately 36,000 Da) consisting of 2 noncovalently bound subunits. The alpha subunit (92-amino acids; "naked" protein MW 10,205 Da) is essentially identical to that of luteinizing hormone (LH), follicle-stimulating hormone, and thyrotropin. The alpha subunit is essential for receptor transactivation. The different beta subunits of the above hormones are transcribed from separate genes, show less homology, and convey the receptor-specificity of the dimeric hormones. The chorionic gonadotropin, beta gene (coding for a 145-amino acid, "naked" protein MW 15,531 Da; glycosylated subunit MW approximately 22,500 Da) is highly homologous to the beta subunit of LH and acts through the same receptor. However, while LH is a classical tropic pituitary hormone, hCG does not usually circulate in significant concentrations. In pregnant primates (including humans), it is synthesized in the placenta and maintains the corpus luteum and, hence, progesterone production, during the first trimester. Thereafter, the placenta produces steroid hormones, diminishing the role of hCG. HCG concentrations fall, leveling off around week 20, significantly above prepregnancy levels. After delivery, miscarriage, or pregnancy termination, hCG levels fall, with a half-life of 24 to 36 hours, until prepregnancy levels are reached.

Outside of pregnancy, hCG may be secreted by abnormal germ cell, placental, or embryonal tissues, in some seminomatous and nonseminomatous testicular tumors; ovarian germ cell tumors; gestational trophoblastic disease (hydatidiform mole and choriocarcinoma); and benign or malignant nontesticular teratomas. Rarely, other tumors, including hepatic, neuroendocrine, breast, ovarian, pancreatic, cervical, and gastric cancers may secrete hCG, usually in relatively modest quantities.

During pathological hCG production, the highly coordinated secretion of alpha and beta subunits of hCG may be disturbed. In addition to secreting intact hCG, tumors may produce disproportionate quantities of free alpha-subunits or, more commonly, free beta-subunits. Assays that detect both intact hCG and free beta-hCG, including the electrochemiluminescent immunoassay assay, tend to be more sensitive in detecting hCG-producing tumors.

With successful treatment of hCG-producing tumors, hCG levels should fall with a half-life of 24 to 36 hours and, eventually, return to the reference range.

The alternate testing method is an enzymatic immunoassay. Values obtained with different assay methods or kits may be different and cannot be used interchangeably.

Reference Values

BETA-HUMAN CHORIONIC GONADOTROPIN, QUANTITATIVE, SERUM
Children(1,2)
Males
Birth-3 months: < or =50 IU/L*
>3 months-<18 years: <1.4 IU/L
Females
Birth-3 months: < or =50 IU/L*
>3 months-<18 years: <1.0 IU/L
Pediatric reference values based on:
1. Chen RJ, Huang SC, Chow SN, Hsieh CY: Human chorionic gonadotropin pattern in maternal circulation. Amniotic fluid and fetal circulation in late pregnancy. J Reprod Med. 1993;38(2):151-154
2. Schneider DT, Calaminus G, Gobel U: Diagnostic value of alpha 1-fetoprotein and beta-human chorionic gonadotropin in infancy and childhood. Pediatr Hematol Oncol. 2001;18(1):11-26
*Human chorionic gonadotropin (hCG), produced in the placenta, partially passes the placental barrier. Newborn serum beta-hCG concentrations are approximately 1/400th of the corresponding maternal serum concentrations, resulting in neonate beta-hCG levels of 10-50 IU/L at birth. Clearance half-life is approximately 2 to 3 days. Therefore, by 3 months of age, levels comparable to adults should be reached.

Adults (97.5th percentile)
Males: <1.4 IU/L
Females
Premenopausal, nonpregnant: <1.0 IU/L
Postmenopausal: <7.0 IU/L

HUMAN CHORIONIC GONADOTROPIN, ALTERNATIVE METHOD
Males
Birth-3 months: Not established
>3 months-49 years: <0.6 IU/L
50 years-80 years: <1.6 IU/L
>80 years: Not established

Females
Birth-3 months: Not established
>3 months-40 years: <0.6 IU/L
41 years-50 years: <6.2 IU/L
51 years-150 years: <7.8 IU/L

Interpretation

Specimens are evaluated for potential heterophile antibody interference in the Roche Elecsys total beta-human chorionic gonadotropin (hCG) immunoassay. Evaluation consists of pretreatment with commercial heterophile antibody blocking tube reagents, serial dilution of the sample, and testing on an alternate platform (Beckman Coulter DxI). The presence of heterophile antibody interference in the Roche Elecsys assay is not suspected when the results from the pretreatment, serial dilution, and the alternative platform agree within 20% of the original result.

The presence of heterophile antibody interference in the Roche Elecsys assay is suspected when 1 or more of the following are observed: a significant decrease in hCG (>20%) upon treatment of the sample with heterophile antibody blocking reagents, lack of linearity upon serial dilutions, or a significant difference in hCG concentration on the alternate platform. When a heterophile antibody interference affecting the Roche Elecsys assay is suspected, the hCG results from this assay are considered false-positive results and should not be used in clinical management.

Heterophile reagent blocking tubes (HBT-Scantibodies) contain a unique blocking reagent composed of specific binders, which inactivate heterophilic antibodies. Once the specific binders have bound to the heterophilic antibodies, the antibodies are no longer able to cause immunoassay interference. Blocking agents do not inhibit all heterophilic antibodies completely and cannot be used to rule out the presence of heterophile antibody interference.

For patients with apparent serum hCG concentrations greater than 15 to 20 IU/L, hCG should also be detectable in urine if it is truly elevated. Failure to detect urinary hCG in such patients can support the suspicion of a false-positive serum hCG test.

After delivery, miscarriage, or pregnancy termination, hCG levels fall with a half-life of 24 to 36 hours, until prepregnancy levels are reached. An absent or significantly slower decline is seen in patients with retained products of conception.

Gestational trophoblastic disease (GTD) is associated with very considerable elevations of hCG, usually above 2 multiples of the median for gestational age persisting, or even rising beyond, the first trimester.

Serum hCG levels are elevated in approximately 40% to 50% of patients with nonseminomatous testicular cancer and 20% to 40% of patients with seminoma. Markedly elevated levels of hCG (>5000 IU/L) are uncommon in patients with pure seminoma and indicate the presence of a mixed testicular cancer.

Ovarian germ cell tumors (approximately 10% of ovarian tumors) display elevated hCG levels in 20% to 50% of cases.

Teratomas in children may overproduce hCG, even when benign, resulting in precocious pseudopuberty. Levels may be elevated to similar levels as seen in testicular cancer.

Among nonreproductive tumors, hepatobiliary tumors (hepatoblastomas, hepatocellular carcinomas, and cholangiocarcinomas) and neuroendocrine tumors (eg, islet cell tumors and carcinoids) are those most frequently associated with hCG production.

Many hCG-producing tumors also produce other embryonic proteins/antigens, in particular alpha fetoprotein (AFP). Therefore, AFP should also be measured in the diagnostic workup of such neoplasms.

Complete therapeutic response in hCG-secreting tumors is characterized by a decline in hCG levels with an apparent half-life of 24 to 36 hours and eventual return to concentrations within the reference range. GTD and some tumors may produce hyperglycosylated hCG with a longer half-life, but an apparent half-life of greater than 3 days suggests the presence of residual hCG-producing tumor tissue.

A rise in hCG levels above the reference range in patients with hCG-producing tumors that had previously been treated successfully suggests possible local or distant metastatic recurrence.

Cautions

Values obtained with different assay methods or kits may be different and cannot be used interchangeably.

Test results cannot be interpreted as absolute evidence for the presence or absence of malignant disease.

This heterophile antibody interference evaluation does not rule out the presence of other interfering substances, such as biotin.

There may be some samples with extremely strong heterophilic interference. In such cases, heterophile-blocking reagents may not be able to block all assay interference.

Despite strenuous efforts at standardization, different human chorionic gonadotropin (hCG) assays show only modest agreements with each other. Therefore, whenever serial monitoring of hCG concentrations is required, the same assay should be used for all measurements.

Transient elevations of serum hCG can occur following chemotherapy in patients with susceptible tumors, due to massive tumor cell lysis; these transient elevations should not be confused with tumor progression.

Normal serum levels of hCG do not always exclude tumor persistence since tumors may undergo transition to differentiated teratomas, which may not produce hCG.

In individuals with incomplete or complete primary hypogonadism, the low sex hormone concentrations may result in elevated hCG due to inadequate negative feedback to the pituitary. In these individuals hCG levels of 3.0 to 5.0 IU/L and, in some cases, as high as 25 IU/L, may be seen. In postmenopausal women, hCG levels ranging from 3.5 to 32 IU/L have been reported. It is recommended that serum luteinizing hormone or follicle stimulating hormone be determined to assess this possibility.

Kidney failure is associated with up to 10-fold elevations in serum hCG levels.

In rare cases, some individuals can develop antibodies to mouse or other animal antibodies (often referred to as human anti-mouse antibodies [HAMA] or heterophile antibodies), which may cause interference in some immunoassays. The presence of antibodies to streptavidin or ruthenium can also rarely occur and may also interfere in this assay. Caution should be used in interpretation of results and the laboratory should be alerted if the result does not correlate with the clinical presentation.

Clinical Reference

1. Cole LA, Khanlian SA, Muller CY: Detection of perimenopause or postmenopause human chorionic gonadotropin: an unnecessary source of alarm. Am J Obstet Gynecol. 2008 Mar;198(3):275.e1-7. doi: 10.1016/j.ajog.2007.09.034
2. Schneider DT, Calaminus G, Gobel U: Diagnostic value of alpha 1-fetoprotein and beta-human chorionic gonadotropin in infancy and childhood. Pediatr Hematol Oncol. 2001 Jan-Feb;18(1):11-26
3. Cole LA, Butler S: Detection of hCG in trophoblastic disease. The USA hCG reference service experience. J Reprod Med.

2002 Jun;47(6):433-444
4. von Eyben FE: Laboratory markers and germ cell tumors. Crit Rev Clin Lab Sci. 2003 Aug;40(4):377-427
5. Sturgeon CM, Duffy MJ, Stenman UH, et al: National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clin Chem. 2008 Dec;54(12):e11-79. doi: 10.1373/clinchem.2008.105601
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7. Sturgeon CM, Viljoen A: Analytical error and interference in immunoassay: minimizing risk. Ann Clin Biochem. 2011 Sep;48(Pt 5):418-432. doi: 10.1258/acb.2011.011073
8. Marks V: False-positive immunoassay results: a multicenter survey of erroneous immunoassay results from assays of 74 analytes in 10 donors from 66 laboratories in seven countries. Clin Chem. 2002 Nov;48(11):2008-2016
9. Tate J, Ward G: Interferences in immunoassay. Clin Biochem Rev. 2004 May;25(2):105-120

Performance

Method Description

The specimen will be evaluated for potential heterophile antibody interference in the Roche Elecsys total beta-human chorionic gonadotropin (hCG) immunoassay. Heterophile Antibody evaluation will consist of pretreatment with commercial heterophile antibody blocking reagents, testing on an alternate platform (Beckman Coulter), and serial dilution of the sample.

Heterophile blocking agents consisted of heterophile blocking tube (HBT)-Scantibodies Inc. for the Roche assay and heterophile blocking agent (HBR)-Scantibodies, Inc. for the Beckman assay. These blockers contain either murine- (HBT) or goat- (HBR) derived proteins in a buffered salt solution.

Beta-hCG, Quantitative, Serum
The Roche hCG assay is a 2-site immunometric sandwich assay using electrochemiluminescence detection. Patient specimen, biotinylated monoclonal hCG-specific antibody, and monoclonal hCG-specific antibody labeled with a ruthenium react to form a complex. Streptavidin-coated microparticles act as the solid phase to which the complex becomes bound. Voltage is applied to the electrode inducing a chemiluminescent emission from the ruthenium, which is then measured against a calibration curve to determine the amount of hCG in the patient specimen.(Package insert: hCG. Roche Diagnostics; V1.0 10/2020)

HCG, Alternative Method
The Access Total beta hCG (5th IS) assay is a sequential two-step immunoenzymatic (“sandwich") assay. A sample is added to a reaction vessel along with a citrate buffer. After an initial incubation, rabbit anti-beta hCG alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-beta hCG complexes are added. The hCG binds to the immobilized monoclonal anti-beta hCG on the solid phase while, at the same time, the rabbit anti-beta hCG alkaline phosphatase conjugate reacts with different antigenic sites on the hCG. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel, and the light generated

by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of total beta hCG in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.(Instruction manual: Access Total beta hCG 5th IS. Beckman Coulter Inc; 2020)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
2 to 4 days

Specimen Retention Time
12 months

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
84702 x 2

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
IEHCG	Interference Eval, Heterophile, HCG	99306-3

Result ID	Test Result Name	Result LOINC® Value
HCGQN	Beta-HCG, Quantitative, S	21198-7
HCGAM	HCG, Alternative Method, S	21198-7
HCGIF	HCG, Interference Heterophile	99307-1

HCGIN	HCG, Interpretation	77202-0