**Review Criteria for Assessment of Professional Use Human Chorionic Gonadotropin (hCG) In Vitro Diagnostic Devices (IVDs)**

This guidance was written prior to the February 27, 1997 implementation of FDA’s Good Guidance Practices, GGP’s. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP’s.

CDRH Final Guidance Cover Sheet

**REVIEW CRITERIA FOR ASSESSMENT OF PROFESSIONAL USE HUMAN CHORIONIC GONADOTROPIN (hCG) IN VITRO DIAGNOSTIC DEVICES (IVDs)**

This is a flexible document representing the current major concerns and suggestions regarding human chorionic gonadotropin (hCG). It is based on 1) current basic science, 2) clinical experience, 3) previous submissions by manufacturers to the Food and Drug Administration (FDA), and 4) the Safe Medical Devices Act of 1990 and regulations in the Code of Federal Regulations (CFR). So that we may revise the draft as necessary, please send your comments to the address given below.

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**DEFINITION:** This generic type device is intended for clinical laboratories (labs) and physician's office labs (POLs) as an IVD test for quantitative and/or qualitative measurement of hCG. Clinical labs can include hospitals and reference labs. POLs can include mini-clinics.

**PRODUCT CODE(S):** JHJ, DHA, JHI

**PANEL:**\*\*\* Clinical Chemistry

**REGULATION NUMBER:** 21 CFR §862.1155

1. Identification. An hCG test system is a device intended for the early detection of pregnancy. It is intended to measure hCG, a placental hormone, in serum, plasma or urine.
2. Classification. Class II (performance standards). 47 FR 4915 - no performance standards established.

**REVIEW REQUIRED:**\*\*\* 510(k)

**PURPOSE:**The purpose of this document is to provide guidance on information to present to the Food and Drug Administration (FDA) before a device may be cleared for marketing. This information enables FDA to make better informed decisions based on a uniform data base. It is our hope that such documents may lead to more reliable, reproducible and standardized commercial tests.

**I. Background**

Pregnancy tests are based on the detection of the hormone hCG in urine or serum. HCG is thought to be produced by trophoblastic tissue and it appears around the 8-9th day after ovulation where fertilization has occurred, or around the 4th day after conception. In a 28 day cycle with ovulation occurring at day 14 hCG can be detected in urine or serum in minute quantities around day 23, or 5 days before the expected menstruation. Its function includes facilitation of implantation as well as maintenance and development of the corpus luteum. The hormone concentration doubles approximately every 2 days and peaks between 7-12 weeks after the first day of the last menstrual period with a mean concentration of 50,000 mIU/mL. Concentrations as high as 100,000 mIU/mL have been reported in normal pregnancies during the first trimester. In normal subjects, hCG in urine provides an early indication of pregnancy. Since elevated hCG levels are also associated with trophoblastic disease and certain nontrophoblastic neoplasms, the possibility of having these diseases must be eliminated before a diagnosis of pregnancy can be made.(1)(2)

HCG is a glycoprotein with a molecular weight of 50,000 daltons. Each molecule consists of an alpha subunit of 18,000 daltons and a beta subunit of 32,000 daltons. HCG, like other glycoprotein hormones, luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) consists of two subunits, alpha and beta. Alpha subunits of these various glycoprotein hormones are structurally very similar, but beta subunits differ in amino acid sequences. These differences are responsible for their biological and immunological specificity.(3)

Human chorionic gonadotropin circulates as the intact molecule in the serum of women who have an uncomplicated pregnancy. Subunits are cleaved rapidly and cleared promptly by the kidney. Thus, urine contains intact hCG, and alpha and beta subunits; however, only intact hCG and beta subunits retain immunologic specificity in urine. The relative concentrations of intact and subunit hCG in serum and urine vary a great deal in patients with trophoblastic disease because of unbalanced synthesis of subunits.(4)

The International Reference Preparation for Chorionic Gonadotropin (1st IRP for CG), now officially designated the 3rd International Standard (3rd IS), contains only pure intact hCG. The 2nd IS contains a mixture of intact hCG and its beta subunit. The 1st IRP is also available as pure beta subunit and pure alpha subunit. It is now possible for manufacturers to characterize and define the specificity of their assays for intact hCG and/or its subunits. It is not possible to obtain uniformity in the reporting of results solely through the use of a common standard since hCG is a complex molecule. Different immunoassays may use different antibodies that recognize different components (epitopes) of the molecule.(5)

It is important for manufacturers to adhere to the WHO guidelines on the labeling of the kits and to specify whether the assay system measures intact hCG, or subunits, or both. This assists obstetricians in interpreting laboratory results.(6)

**II. Description**

1. **Historical Background**

The first clinically useful bioassay was introduced by Ascheim (1927) and by Zondek (1931) and was characterized by enlargement and luteinization of the corpus luteum of the immature mouse following injections of urine from normally pregnant women. Zondek noted similar results when the urine from women with choriocarcinoma or ovarian cancer, or from men with testicular neoplasms, were used. The assays were followed by the Friedman test (1931) and the Xenopus laevis test (Shapiro, 1934), which utilized urine from pregnant women with the end point being ovulation in the rabbit and South African toad, respectively. The first tests could confirm pregnancy approximately two months after a missed period.(7) The introduction of hemagglutination and latex agglutination methods simplified testing and improved early pregnancy detection. However, analyte sensitivity was limited to 150-1000 mIU/mL. Bioassays were replaced by immunoassays with the first being reported in 1960. Sensitive radioimmunoassay and immunoradiometric (IRMA), and enzyme immunoassay (EIA) techniques now available allow an accurate and precise quantitation of hCG through the use of highly specific antibodies. These assays can detect pregnancy by the first day of the missed menstrual period. The development of monoclonal antibodies to hCG provides a supply of homogeneous antibodies and improves the specificity of the assay. (8)(9)

**III. Performance Characteristics/Laboratory Evaluation**

The FDA requires different types and amounts of data and statistical analyses to be included in a manufacturer's application for marketing of IVDs depending on whether the test is a quantitative or qualitative assay and the site of intended use.

Performance characteristics must be demonstrated for all sample types/matrices claimed for use or which demonstrate statistical differences. Specimen and protocol:

¶ Whole blood specimens should be collected into suitable tubes with/without anticoagulants, allowed to clot at room temperature and centrifuged. All specimens not tested within 48 hours of collection should be stored at -20 ° C. Repeated freezing and thawing should be avoided. Serum specimens showing gross hemolysis, gross lipemia or turbidity may give false results.

¶ A first morning ***urine*** is recommended because hCG concentration is highest at this time. If specimens cannot be assayed immediately, they should be stored at 2-8 °C for up to 48 hours.(10)

Note: For qualitative urine assays making claims for use any time of day, it is recommended that samples collected any time of day be used in the study.

1. **Comparison studies**
   1. Accuracy as determined by correlation of the proposed device with a currently marketed device

CLINICAL LABORATORIES

It is recommended that 40 or more fresh serum specimens be collected. For quantitative tests, include specimens with hCG concentrations distributed throughout the assay range, as well as samples in which hCG is not detectable. For qualitative tests, it is strongly recommended that both positive and negative specimens be included. It is preferable if an evenly distributed number of specimens positive and negative for hCG, including some close to the cut-off, are included.

It is recommended that 40 or more urine samples be collected. It is preferable if an evenly distributed number of specimens both positive and negative for hCG are included. FDA realizes that this may not be possible with random sampling.

POL

It is recommended that 40 or more specimens be analyzed at each of the 3 POL sites. If the kit utilizes more than one specimen matrix, e.g., serum and urine, it is recommended that a total of 40 specimens which include an equal number of each matrix be analyzed. Consider including an evenly distributed number of positive and negative specimens. Describe the type of practice and the background/training of personnel (e.g., physician, nurse, clinical assistant) performing the tests. Also include this information in the Performance Characteristics section of the package insert.

For spiked specimens, FDA recommends including specimens around (e.g., 10-20% above and below) the sensitivity level. Also, FDA requests that the concentrations of spiked specimens be provided in the 510(k) file.

* 1. Accuracy may be determined by dilution and recovery studies as appropriate.
  2. Data Analysis

**Quantitative**

FDA recommends analyzing the data using linear regression. FDA also recommends reporting the slope, intercept, correlation coefficient, and assay range. It is preferable to plot the data as well.

**Qualitative/POL**

The data can be expressed in terms of percent (%) agreement; data from all sites may be summarized. FDA requests that the types of samples (e.g., serum, urine from pregnant, non-pregnant, post menopausal individuals, etc.) be described.

1. **Specificity**

It is recommended that specificity studies be performed on specimens with high physiological concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). High levels of LH should not significantly cross-react with the hCG antibody used. Similar studies may also be performed with human placental lactogen (hPL) and human growth hormone (hGH). Spiking of samples may be necessary.

1. **Interfering Substances**

It is recommended that interference studies be performed with prescription/OTC drugs, elevated levels of urine/serum analytes (e.g., glucose, protein, albumin, bilirubin, hemoglobin), and urine pH.

1. **Sensitivity/Detection Limit**

**Quantitative:** The sensitivity/detection limit is the smallest single result which, with stated probability (nominally 95%), can be distinguished from a suitable blank. It is defined as two times the standard deviation of a suitable blank, e.g., the zero standard, based on 20 consecutive replicate tests.

**Qualitative:** The sensitivity/detection limit is the analyte concentration at which 95 percent (%) of the test results are positive. Assay sensitivity should be such that small quantities of hCG will be detected while false-positive results due to the presence of LH will be minimized.(2) Additionally, it is expected that the 95% will have reacted within the specified time frame.

Sensitivity can be evaluated by spiking at least 20 clinical samples from normal, nonpregnant females or males with five different concentrations of hCG below, at and above stated sensitivity. For example, use 0, 20, 25, 50, 100 mIU/mL for a kit with a detection limit of 25 mIU/mL. FDA requests that the concentrations tested be provided for the 510(k) file.

1. **Imprecision studies (Quantitative)**

FDA recommends following the NCCLS guidelines (EP5-T2) or an alternate scientifically valid protocol when evaluating imprecision.

Data for within-run and total precision should be summarized separately. FDA recommends reporting the number of assays run, mean, standard deviation and coefficient of variation (CV) for each level of sample in the performance characteristics section of the package insert.

1. **Expected Values**

The manufacturer may state that the test is capable of detecting pregnancy at a level consistent with that of the first day of a missed period and no sooner, unless validated by clinical data. The manufacturer may also discuss varying hCG levels such as, undetectable levels, levels after conception and implantation, levels during the first trimester. References should be provided as needed.

1. **Calibration**

The source of reference material that the standards or test are calibrated against (1st IRP, 2nd IS or 3rd IS) for hCG should be stated in the package insert.

1. **Quality Control**

The assayed quality control (QC) materials provided with the device or recommended for use should state the levels of hCG. It is recommended that positive QC materials used with qualitative assays be near the stated sensitivity of the assay to aid in interpretation of weak positive results.

The insert should not include specific recommendations for the frequency for which external quality control samples are used. This determination should be left to the discretion of the laboratory in conformance with their local, state, and/or federal accreditation requirements.

When appropriate, manufacturers may recommend frequencies for use of particular device design components (e.g., frequencies for QC checks). If these recommendations are made, it should be clear that these frequencies apply only to assessment of device function per se and cannot be used to evaluate the total analytical performance of the test. These recommendations should be accompanied by a clear disclaim indicating that QC requirements should be performed in conformance with local, state, and/or federal accreditation requirements.

It is also recommended that instructions for actions with invalid results be included in the package insert.

Additionally, the sensitivity (detection limit) of a reference line and the components of the control bar/line are requested for the 510(k) file.

1. **Stability Data**

The data showing stability of the reagents need not be submitted to the FDA, but must be kept on file by the manufacturer in accordance with Good Manufacturing Practice (GMP) requirements. A summary of data, including a description of the stability protocol, for calibrators and controls (if included in the kit) are requested for the 510(k) file.

1. **Limitations**

The list of examples presented here is not all inclusive. Limitations should be included in the labeling (i.e., package insert) **as appropriate** to the end user (e.g., clinical lab, POL) and/or specimen type.

* 1. Specimens that have been contaminated with radioactivity may give erroneous results.
  2. The "High-dose hook" effect should be described and explained.
  3. Trophoblastic or nontrophoblastic neoplastic conditions should be ruled out before reporting results.(11)
  4. Samples from patients on chemotherapy for cancer should be ruled out before running the assay.
  5. Grossly hemolyzed or lipemic samples should not be used since they may give inaccurately lower or erratic results.
  6. Elevated levels have been detected in the serum and urine of patients with tumors of the placenta and choriocarcinomas.(12)
  7. Low titer elevations of hCG can occur in normal, nonpregnant subjects.(13)
  8. This kit is not intended for any use other than early detection of pregnancy.
  9. Ectopic pregnancy cannot be distinguished from normal pregnancy from hCG measurements alone.
  10. Positive hCG levels may be detectable for several weeks following delivery or abortion.
  11. Specimens testing positive during the initial days after conception may be negative later due to natural termination of the pregnancy.
  12. As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample.

**IV. Labeling**

The package insert should be concise, easy to understand and contain clear illustrations and drawings. The labeling format should conform to the CFR 809.10 labeling regulations.

**V. Bibliography**

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4. Lab Report for Physicians. Standardization of human chorionic gonadotropin. December 1985; 7:12.
5. Jeffcoat, SL. Standardisation of hCG immunoassays and pregnancy kits. Lancet 1982; 1:803.
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7. Chayen J, Daly JR, Loveridge N. The cytochemical bioassay of hormones. Recent Prog. Horm. Res. 1976; 32:33-72.
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10. Krieg AF. Pregnancy tests and evaluation of placental function. Clinical Diagnosis and Management by Laboratory Methods, 18th edition, Henry JB, editor, 1979; 680.
11. Braunstein GD, Vaitukaitis JL. Carbone PP and Ross GT. Ectopic production of human chorionic gonadotropin by neoplasms. Ann Intern. Med. 1973, 78: 39-45.
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ABBREVIATIONS

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| CDRH | Center for Devices and Radiological Health |
| CLIA | Clinical Laboratory Improvement Act |
| DCLD | Division of Clinical Laboratory Devices |
| FDA | Food and Drug Administration |
| FSH | Follicle Stimulating Hormone |
| HCG | Human Chorionic Gonadotropin |
| HGH | Human Growth Hormone |
| HPL | Human Placental Lactogen |
| IRP | International Reference Preparation |
| IS | International Standard |
| IVD | In Vitro Diagnostic |
| LH | Luteinizing Hormone |
| NCCLS | National Committee for Clinical Laboratory Standards |
| POL | Physician's Office Laboratory |
| QC | Quality Control |
| TSH | Thyroid Stimulating Hormone |

CHECKLIST

Instructions: Use this checklist for premarket submissions for human chorionic gonadotropin (hCG)/pregnancy tests intended for use in clinical labs and POLs. Please check the box next to the items below that you have included in the premarket notification. Please note that some are required while others are only suggested. Those required are identified as such.

* CDRH Premarket Submission Cover Sheet
* Truthful and Accurate statement verbatim as required by 21 CFR 807.87(j). Additions and deletions are not permitted.
* 510(k) summary **or** statement as required by 21 CFR 807.92 or 21 CFR 807.93 respectively.
* Sensitivity/Detection Limit Data
* Specificity Data including LH, FSH, and TSH
* Interfering Substances Data
* Comparative Study Data including the following, where applicable: summary of study protocol, range of samples, concentrations of spiked specimens used, names and addresses of POL sites, and the background/training of persons performing tests.
* Antibody characterization/purification information
* Predicate Device Labeling
* Indications for Use form