

# Immunoassay

**REF** CMN0301 / CMN0302 / CMN0303 / CMN0304 / CMN0305

50 tests\*1 / 100 tests\*1 / 100 tests\*2 / 100 tests\*5 / 50 tests\*2

## $\beta$ -hCG CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of  $\beta$ -hCG ( $\beta$ -Human Chorionic Gonadotropin) in human serum.

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### Key to Graphical Symbols Used

**LOT**

batch code



use by



manufacturer



contains sufficient for <n> tests

**IVD**

*in vitro* diagnostic medical device



temperature limitation

**REF**

catalogue number



consult instructions for use

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## Introduction

Human Chorionic Gonadotropin is a glycoprotein hormone produced by trophoblast during pregnancy, has a similar structure to the pituitary hormones FSH, TSH and LH. The  $\alpha$ -subunit of hCG is identical with the  $\alpha$ -subunit of LH, but the  $\beta$ -subunit is different and exhibits different immunity and biological activity [1-3].

Determination of serum  $\beta$ -hCG can be used for early pregnancy diagnosis and pregnancy monitoring. Serum and urine concentrations of biologically active hCG rise exponentially in the first trimester of pregnancy, doubling every 48h, to a peak at about 10 weeks of gestation and then slowly decrease until the end of pregnancy. After the end of pregnancy, hCG concentration decrease back to  $<5$  mIU / mL [4].

In general, ectopic pregnancy and threatened abortion has a lower  $\beta$ -hCG levels or growth rate than normal pregnancy; multiple pregnancies have a higher  $\beta$ -hCG level than normal pregnancy. The monitoring of serum  $\beta$ -hCG levels contribute to threatened abortion, ectopic pregnancy diagnosis.

## Measurement Principle

This assay is based upon the two-step sandwich method. In the first step, the sample and  $\beta$ -hCG antibody coated microparticles are combined. During the incubation,  $\beta$ -hCG present in the sample binds to the antibodies coated microparticles. After the washing, in the second step, enzyme conjugate is added to the reaction mixture. During the incubation, a complex is generated among the microparticles, the  $\beta$ -hCG within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLU. The RLU is proportional to the concentration of  $\beta$ -hCG in the patient sample.

## Materials provided

### 1. Calibrators

7 vials lyophilized Calibrator A through G. The matrix is PBS buffer containing casein. Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Invert the calibrator several times to mix it completely. Then allow the reconstituted material to stand for at least 30 minutes.

### 2. Reagent pack

Reagent pack provided ready to use.

	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Sample Diluent	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

### ● Microparticles Solution

$\beta$ -hCG antibody coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives.

### ● Enzyme Conjugate

Horseradish-peroxidase labeled anti- $\beta$ -hCG in PBS buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

### ● Sample Diluent

PBS buffer containing casein. Contains a selection of preservatives.

## Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

## Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

## Metrological Traceability of Calibrators

The measurand or analyte in the  $\beta$ -hCG calibrators is traceable to a calibrator purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products), China.

## Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

## Storage

1. Store the kit at 2-8°C. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10°C for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10°C for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10°C in an upright position.
4. Seal and return the remaining calibrators at 2-8 °C immediately after the experiment, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C, under which conditions the stability will be retained for 2 months. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 3 cycles.

## Sample

1. Collect serum samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
7. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
8. Avoid grossly hemolytic, lipemic or turbid samples.
9. Cap and store the samples at 18-25°C for no more than 8 hours, for longer use samples should be capped and stored at 2-8°C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20°C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
10. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
11. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
12. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
13. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

## Measurement Procedure

1. **Check the consumable materials**
  - Verify adequate volume of consumable materials is present prior to running the test.
  - Refer to the Assay Analyzer's operation manual.
2. **Load the kit**
  - Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
  - Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
  - If the bar code cannot be read in exceptional cases, they can be recognized manually.
  - Refer to the Assay Analyzer's operation manual.
3. **Order tests**
  - Place the sample tubes or cups on the sample rack, 25  $\mu$ L of samples for each test. But consider the sample container and 150  $\mu$ L of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.

- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of  $\beta$ -hCG antigens in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

### 4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

### 5. Dilute the sample

Samples with  $\beta$ -hCG value exceeding 2000 mIU/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

- The concentration of the sample after dilution should not be less than 15 mIU/mL.

## Measurement Results

The sample test results are determined automatically by the system software. The amount of  $\beta$ -hCG antigen in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

## Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the acceptable ranges. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

## Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
4. Accurate calculation of gestational weeks can get accurate risk assessment results.
5. Different populations may have different median, the detection rate of abnormal pregnancy may vary depending on the number of samples.
6. This test measures concentrations within the range of 2-2000 mIU/mL. If  $\beta$ -hCG concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the recommended dilution is 1:99 of this test, under this condition, allowing samples to be up approximately 200000 mIU/mL.

## Biological Reference Interval

A total of 1017 samples of healthy women without pregnancy were examined. The recommended normal range (95<sup>th</sup> percentile) of  $\beta$ -hCG in healthy women without pregnancy is <5.0 mIU/mL.

It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

## Performance Characteristics

### 1. Measurement Precision

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (mIU/mL)	Within-run	Total
			%CV	%CV
1	80	6.30	4.84	8.31
2	80	17.56	3.05	6.16
3	80	412.02	3.44	5.73

\*Representative data; results in individual laboratories may vary from these data.

### 2. Analytical Sensitivity

Limit of Blank: 1.0 mIU/mL

Limit of Detection: 2.0 mIU/mL

Limit of Quantitation: 3.5 mIU/mL with a coefficient of variation of  $\leq$  20 %

### 3. Analytical Specificity

**Cross reaction:** the following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration
hTSH	520 $\mu$ IU/mL
hFSH	290 IU/L
hLH	200 IU/L

**Interference:** No interference with 1000 mg/dL of haemoglobin, 24 mg/dL of bilirubin and 3000 mg/dL triglyceride.

### 4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a  $\beta$ -hCG reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	424	10.99	0.9764	0.957

## Literature References

1. Cole LA, Immunoassay of human chorionic gonadotropin, its free subunits and metabolites [J], Clin Chem.1997,43(12):2233~2243.
2. Birken, S. Chemistry of human choriogonadotropin. Annales Endocrinologie 1984; 45: 297-305.
3. Human reproduction unit, WHO report of meeting. Assay of protein hormones related to human reproduction: problems of specificity of assay methods and reference standards. Acta Endocrinology, 1972; 71: 625-637.
4. Vaitukaitis, J.L., Recent Progress in Hormone Research, 1976; 32: 289.