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Immunoassay

REF **CMF0201/CMF0202/CMF0203/CMF0204/CMF0205**

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

FSH CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of FSH (Follicle-Stimulating Hormone) concentration in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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Introduction

FSH (follicle-stimulating hormone) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland.

A follicle-stimulating hormone test measures the amount of FSH in a blood sample. FSH is produced by the pituitary gland.

FSH is a glycoprotein gonadotropin secreted by the anterior pituitary in response to GnRH (gonadotropin-releasing hormone), which is released by the hypothalamus. The same pituitary cell also secretes LH (luteinizing hormone). FSH and LH are composed of alpha and beta subunits. The specific beta subunit confers the unique biologic activity. FSH and LH bind to receptors in the testis and ovary and regulate gonadal function by promoting sex steroid production and gametogenesis.[1-3]

In women, FSH helps control the menstrual cycle and the production of ovums by the ovaries. The amount of FSH varies throughout a woman's menstrual cycle and is highest just before she releases an ovum.

In men, FSH helps control the production of sperm. The amount of FSH in men normally remains constant.

It has been found that recombinant human FSH may affect some improvement by either providing sperm in ejaculate or increasing intracytoplasmic sperm injection success in infertile men with maturation arrest.[4]

In females and males, FSH and LH are ordered as part of the workup of infertility and pituitary or gonadal disorders. FSH may be ordered when a woman's menstrual cycle has stopped or become irregular, to determine if the woman has entered menopause. In women, FSH and LH levels can help to differentiate between primary ovarian failure (failure of the ovaries themselves) and secondary ovarian failure (failure of the ovaries due to disorders of either the pituitary or the hypothalamus). Increased levels of FSH and LH are consistent with primary ovarian failure.[5,6]

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, anti-FSH coated microparticles and enzyme labeled anti-FSH are combined. FSH present in the sample is allowed to react simultaneously with the two antibodies, resulting in the FSH being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is generated between the solid phase, the FSH within the sample and enzyme-linked antibodies by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of FSH in the samples.

Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing bovine serum and BSA (bovine serum albumin). Contains a selection of preservatives.

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Mouse monoclonal anti-FSH coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled mouse monoclonal anti-FSH in Tris-HCl buffer containing bovine serum. 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 Analyzer which is 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 cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water

Metrological Traceability of Calibrators

The product calibrators are manufactured using lyophilized FSH powder and signal matched to our working calibrators, which are also signal matched to a higher order calibrator purchased from WHO (The World Health Organization) 2nd IRP # 78/549, at each concentration level.

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. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and syphilis. It is recommended that all materials of human origin be considered potentially infectious. 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℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ 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 ℃ in an upright position.
4. Seal and return the remaining calibrators to 2-8℃, under which conditions the stability will be retained for 2 months, for longer use, store opened calibrators in aliquots and freeze at -20℃. Avoid multiple freeze-thaw 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. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. 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.
9. 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.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. 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.
12. 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 cups or tubes on the sample rack, 25 μL of samples for each test. But considering the sample container and 150 μL 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..
- 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. The instrument perform 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 Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of FSH 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 cups or tubes 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 instrument are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a FSH value exceeding 160 mIU/mL may be diluted manually. Calibrator A is used to dilute the samples.

After dilution, multiply the result by the dilution factor.

- The concentration of the sample after dilution should not be less than 2.5 mIU/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of FSH 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 the stored results.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

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. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
5. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
6. In certain cases of infertility, treatment with human gonadotropins poses a potential problem for the accurate measurement of FSH levels. The FSH that is administered can cause the patient to produce antibodies to FSH which will interfere directly with the assay.
7. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
8. This test measures concentrations within the range of 1.0-160 mIU/mL. If FSH concentrations above the measuring range to be expected, it is recommended to dilute samples with Calibrator A, the maximum dilution is 1:4 of this test, allowing samples to be quantitated up to approximately 800 mIU/mL.

Biological Reference Interval

The suggested normal range (central 95% interval) was obtained by testing serum samples from 120 normal males, 45 normal cycling females and 52 postmenopausal females. For this study, the follicular phase was defined as the period of time from 10 to 4 days prior to the mid-cycle peak. The luteal phase was defined as the period of time from 4 to 10 days following the mid-cycle peak. Cycle days were synchronized to the mid-cycle peak, the day on which the FSH concentration was most elevated. The results are presented in the following table. 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.

		N	Mean Value (mIU/mL)	Reference Interval (mIU/mL)
Males		120	5.6	1-12.1
Normally Menstruating Females	Follicular Phase	145	4.5	2.5-11.4
	Mid-cycle Peak	45	8.1	3.3-21.7
	Luteal Phase	140	3.65	1.2-7.0
Postmenopausal Females		52	62.12	18.8-132

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run	Total
			%CV	%CV
1	80	9.28	2.55	3.46
2	80	25.47	2.75	3.73
3	80	44.28	2.04	3.42

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

2. Analytical Sensitivity

Limit of Blank=0.05mIU/mL
Limit of Detection=0.12mIU/mL
Limit of Quantitation=0.2mIU/mL with a coefficient of variation of ≤ 20%.

3. Analytical Specificity

Cross reaction: this assay is designed to have an analytical specificity of less than 0.3 mIU/mL cross reactivity with the substances listed below, at the concentration levels listed, in Tris-NaCl buffer containing bovine serum and BSA and found no cross reaction with the test:

Substances	Concentration	Cross reactivity
HCG	22800 mIU/mL	＜0.3 mIU/mL
LH	500 mIU/mL	＜0.3 mIU/mL
TSH	500 μIU/mL	＜0.3 mIU/mL

Interference: this assay is designed to have no interference with the substances listed below, at the concentration levels listed, in serum samples.

Interferent	Concentration
Bilirubin	20 mg/dL
Hemoglobin	3000 mg/dL
Triglyceride	3000 mg/dL

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a FSH assay which was already CE marked. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient

Linear Regression	324	0.4170	1.0802	0.9908

5. High Dose Hook Effect

The high-dose hook effect was determined by addition FSH to the human serum up to a maximum of 23179.08 mIU/mL. Whenever samples containing extremely high analyte concentrations are tested, the high-dose hook effect can mimic concentrations lower than real. Analysis of high-dose hook effect was evaluated by testing one high-concentration FSH-spiked sample. The sample resulted in a calculated concentration value above the assay range, indicating no sample misclassification.

Literature References

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