PROBLEM	POSSIBLE CAUSE	SOLUTION
2) High E2 test results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer test specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.
3) Low E2 test results	a) Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong sample identification.	Mark the sample I.D. at the time of sample collection.

in vitro diagnostic Reagent, not for medicinal use

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E2 (ESTRADIOL) *i*Clia

Chemiluminesence Immunoassay for the Quantitative determination of Estradiol (E2) in Human Serum/ Plasma

1. INTRODUCTION

Estradiol (E2) is the most bioactive estrogen, which is mainly secreted by the ovaries, and can also be secreted in small amounts by the adrenal glands and testes of men. Ninety-eight percent of the circulating blood is bound to albumin and SHBG, and only a small amount is present in the free state. E2 mainly promotes the growth of female reproductive epithelium, breast, uterus and long bones and the development of secondary sexual characteristics, participates in lipid metabolism, regulates many functions of vascular smooth muscle cells and endothelial cells, and plays a central role in the control mechanism of ovulation. Lack of E2 will lead to amenorrhea, genital atrophy and osteoporosis and cardiovascular disease, etc., can affect the development of secondary sexual characteristics in girls before pubertal development. At the same time, abnormally high estradiol (E2) levels in men indicate feminization syndrome.

2. INTENDED USE

E2 (Estradiol) iClia is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of estradiol (E2) in human serum/plasma, as an aid in the diagnosis of ovarian disease in conjunction with other laboratory and clinical findings. The assay kit is intended for in vitro diagnostic use & is only operational in conjuction with J. Mitra CLIA Analyzer.

3. PRINCIPLE

E2 (Estradiol) iClia is chemiluminescent immunoassay based on the "competitive" principle. The magnetic microparticles are coated with anti-E2 antibodies. The assay buffer, anti-E2 antibodies coated magnetic microparticles and samples are mixed and incubated in an assay cup. After incubation, E2 labelled AE conjugate is added to reaction mixture. During incubation AE labelled E2 and E2 in sample compete for the binding site of E2 antibody on the binding magnetic particle, and the Microparticle-E2 antibody/E2 immune complex is kept with the help of a magnetic separator. Unbound acridinium ester and other substance are removed by washing. After washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative Light units (RLUs). There is an indirect relationship between the amount of E2 present in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the established calibration curve and E2 concentration in the sample is expressed as pg/mL.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 50 Tests
- 100 Tests

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer (RA)	Magnetic microparticles coated with anti-E2 antibodies as preservatives.
Assay Buffer (RB)	Buffer containing protein stabilizer and antimicrobial agent as preservatives.
AE Conjugate (RD)	E2 antigen linked to acridinium ester with protein stabilizers.
Calibrator-1 (C0)	Low concentration of E2 of in Human Serum containing preservatives.
Calibrator-2 (C1)	High concentration of E2 of in Human Serum containing preservatives.
Control-1 (Q1)	Low concentration of E2 of in Human Serum containing preservatives.
Control-2 (Q2)	High concentration of E2 of in Human Serum containing preservatives.
Reagent Plugs	Silicon caps to cover the opened reagents.

7. STORAGE AND STABILITY

The kit should be stored at 2-8ºC in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8ºC.**

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- **Pre-Trigger Solution:** Hydrogen peroxide solution.
- **Trigger Solution:** Sodium hydroxide solution.
- **Wash Buffer:** Phosphate buffered saline solution with surfactant.
- **Assay Cup**
- **J. Mitra CLIA Analyzer**

All materials and analyzer to be used for running the E2 iClia shall be from J. Mitra & Co. Pvt. Ltd.

9. SPECIMEN COLLECTION & HANDLING

1. Only human serum or plasma samples should be used for the test.
2. For serum collection use serum vacutainer. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
3. For plasma collection: use Dipotassium EDTA, Tripotassium EDTA, Sodium heparin and lithium heparin gel vacutainer.
4. Specimens should be free of microbial contamination and may be stored at 2-8ºC for one week, or frozen at -20ºC or lower. Avoid repeated freezing and thawing.
5. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
7. Always use clear specimens. Centrifuge viscus/ thick or turbid specimen at 10,000 RPM for 15 minutes or 5,000 RPM for 30 minutes prior to use to avoid inconsistent result.
8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

E2 iClia test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. Centrifuge the sample at 10,000 rpm for 15 minutes or 5,000 rpm for 30 minutes.

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8ºC.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microparticles that may have settled during transport or storage.
5 Once reagents are opened, reagent plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugs are not used according to the instructions given.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7 To avoid contamination, wear clean gloves when placing a reagent plug on an uncapped reagent bottle.
8. Once a reagent plug has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
9. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8°C (with Reagent plugs) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent plugs placed) while in refrigerated storage off the system, the reagent kit must be discarded.
10. Run control-1 & control-2 in each assay to evaluate validity of the kit.
11. Distilled or deionised water must be used for wash buffer preparation.
12. Avoid strong light exposure during the assay.
13. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia Analyzer user manual for detailed information on preparing the analyzer.
2. Before loading the E2 iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microspheres that may have settled during transporation/ storage. Once the microspheres have been loaded, no further mixing is required.

   Important Note: Swirl the microparticle (RA) bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. *Once the microspheres have been resuspended, remove the cap and place the the reagent plug on the bottle to make it ready to use. Remove the cap of (RA), (RB) and (RD) bottles and place the reagent plugs before use as follow*

   (RA) & (RB) : Natural color plug

   (RD) : Brown color plug
3. Load the E2 iClia reagent kit on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 μL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 30 μl.
7. Ensure sample positons are properly define at the time of loading in the analyzer.
8. The E2 test-specific parameters are stored in barcode placed on the reagent tray and read through barcode reader. In cases, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
9. Mix E2 (Estradiol) iClia calibrators and controls by gentle inversion before use. Open the cap and place the calibrator-1, calibrator-2, control-1 and control-2 vials into each respective sample positions. Read the barcode for calibrator and controls provided with the kit.
10. Run calibration as mentioned in heading calibration below.
11. Press Run. The test result for first sample will be obtained at 30 minutes.
12. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Calibration

1. Traceability: This assay has been standardized against the standard; NRCCRM, NIM-RM3603).
2. Every E2 (Estradiol) iClia kit has a two-dimension code label containing the predefined master curve of the particular reagent lot.
3. Test both the Calibrators in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that controls values are within the validity range specified in the E2 iClia QC data sheet.
4. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless:
5. Recalibrate the analyzer in following conditions:
   a) After each exchange/use of new lot (Test reagent and Pre-trigger/ Trigger solution/wash buffer).
   b) Every 15 days or at the time of any component to be changed.
   c) Controls are out of validation range.
   d) Required by pertinent regulations.
   e) After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the E2 (Estradiol) iClia.

14. RESULT CALCULATION:

The analyzer automatically calculates the concentrations of each sample. The results are given in pg/ml.

a) RESULT INTERPRETATION

If sample concentration is lower than the lower limit of the linear range, report the result <15.00 pg/mL, while > 2400 pg/mL when it is higher than the upper limit of linear range. Sample with concentration higher than linear range can be further diluted 1:5 or 1:10 with Sample diluent.

For the calculation of the concentration, this dilution factor has to be taken into account.

Determination of Reference Interval

Reference Interval of E2 (Estradiol) iClia is as follows, which is established referring to literatures, based on the rest results of more than 60 clinical samples.

Tests for Male		<65 pg/mL
Tests for Female	Follicular Phottom	<170 pg/mL
	Mid-menstrual period	50 - 480 pg/mL
	Luteal phase	28 - 230 pg/mL
	Post-menopause	<55 pg/mL

Each laboratory should establish its own range of normal value. The values given above are only indicative. Due to the differences in geography, race, gender or age, it is suggested that each laboratory establish its own reference interval or conduct verification of the existing reference interval.

15. PERFORMANCE CHARACTERISTICS

- Assay results obtained in individual laboratories may vary from data presented in this product insert.

Limit of Blank (LoB)

- The Limit of Blank was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
- The Limit of Blank is the 95th percentile value from n >20 measurements of analyte free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%.
- The observed LoB value was ≤10 pg/ml.

Accuracy: The accuracy of E2 (Estradiol) iClia was detected with 60 clinical specimen and compared with Roche CLIA. The co-relation co-efficient is ≥0.980.

Precision

Intra Assay Variation

Within run variation was determined by 10 replicate measurements of two different E2 control sera(Low) and (High) in one assay in 3 different lots. The within assay variability is <10 %.

Inter Assay Variation

Between run variation was determined by 10 replicate measurements in 10 sequential days of two different control sera (Low) and (High) in 3 different lots. The between assay variability is <15.0%.

Linearity

The linearity was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP6-A requirements.

The linearity range was verified by more than 6 concentration levels which encompass or be equal to the minimum and the maximum values of linearity range and duplicate assays in triplicate in single run for each lot at all 6 levels.

The E2 (Estradiol) iClia kit has been demonstrated to be linear from 15 pg/ml to 2400 pg/ml, regression (R^2) of more than >0.990.

Specificity

Interference

A study was performed based on guidance from CLSI EP7-A2.

Potentially interfering substances were evaluated to determine whether E2 concentrations were affected when using the E2 (Estradiol) iClia kit. Samples containing the potential interferents were prepared at two E2 concentrations. The samples were assayed, and the E2 concentrations of the spiked samples were compared to the reference samples.

Potential Interferent	Interferent Concentration	% Interferent Bias
Bilirubin	20 mg/dL	<10%
Hb	500 mg/dL	<10%
Intralipid	1000 mg/dL	<10%
Total protein	10 g/dL	<10%
RF	1000IU/mL	< 10%
ANA	400AU/mL	< 10%
HAMA	600ng/mL	< 10%

16. LIMITATION OF THE TEST

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the E2 results are inconsistent with clinical evidence, additional testing is recommended.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.
- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Additional information may be required for diagnosis.
- The E2 (Estradiol) iClia assay kit is susceptible to interference effects from triglycerides at > 500 mg/dL.

17. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

18. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls/ Calibrator deterioration due to improper storage or used after expiry.	Ensure calibration is done after 15 days and use controls/ Calibrator within 30 days once opened and check storage temp. It should be 2-8ºC.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8ºC.
	d) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.