PROBLEM	POSSIBLE CAUSE	SOLUTION
	c) Reagents deterioration due 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 microspheres by gentle shaking/ inversion before use.
2) High AFP test results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer 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 microspheres by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.
3) Low AFP 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 microspheres by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.

in vitro diagnostic Reagent, not for medicinal use

VER-01 R-00

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MIN:CAF145
Rev. Date: Jan.-25

AFP *i*Clia

Chemiluminesence Immunoassay for the quantitative measurement of Alpha Fetoprotein (AFP) in human serum/plasma

1. INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein that is synthesized mainly in the liver of the fetus. At 13 weeks, AFP accounts for 1/3 of total plasma protein. It peaks at 30 weeks of gestation and declines gradually thereafter. At birth, the plasma concentration is about 1% of the peak, about 40mg/L. Close to adult level (less than 30i g/L) at one year of age. In fetuses with neural tube defects, spina bifida, and anencephaly, AFP may enter the amniotic fluid through the open neural tube, resulting in a significant increase in AFP content in the amniotic fluid. When fetal death in the uterine cavity, teratoma and other birth defects, the AMniotic fluid AFP can enter the maternal blood circulation through the amniotic fluid. In 85% of mothers with spina bifida and anencephaly, the elevation of plasma AFP at 16-18 weeks of gestation is diagnostic, but must be combined with clinical experience to avoid false positive errors. In adults, when liver cells become cancerous, tumor cells can resume their AFP production function. As the disease progresses, the level of AFP in the serum increases sharply. AFP may also be elevated to varying degrees in other gastrointestinal tumors, such as pancreatic or lung cancer. AFP detection is mainly used for dynamic monitoring of patients with malignant tumors to assist in judging disease progression or therapeutic effect, and cannot be used as the basis for early diagnosis or diagnosis of malignant tumors, and cannot be used for tumor screening in the general population.

2. INTENDED USE

AFP iClia is intended for the *in vitro* quantitative measurement of alpha fetoprotein (AFP) in human serum/plasma. This kit is only operational in conjuction with J. Mitra CLIA Analyzer.

3. PRINCIPLE

AFP iClia kit is a chemiluminescent based on “Sandwich principle”. The magnetic microparticle are coated with anti-AFP IgG antibodies.

The samples are added in the assay cup containing assay buffer and anti-AFP IgG antibodies coated microspheres followed by addition of AE conjugate (anti-AFP IgG labeled acridinium ester conjugate) to assay cup. A sandwich complex is formed wherein AFP (from serum/plasma sample) is “trapped” or “sandwiched” between the microspheres coated antibody and antibody labelled with AE conjugate. Unbound conjugate is then washed off with the wash buffer. The amount of bound AE conjugate is proportional to the concentration of AFP present in the sample. Finally pre-trigger and trigger solution containing hydrogen peroxide and sodium hydroxide solution is added to reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of AFP present in the sample and RLUs detected by the optical system. Results are calculated automatically based on the established calibration curve.

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 the 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
	Do not use if package is damaged		Keep away from sunlight
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 50 Test Pack
- 100 Test Pack

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer (RA)	Magnetic microspheres coated with Biotin labelled Anti-AFP antibodies with preservatives.
Assay Buffer (RB)	Buffer containing 8-Anilino-1-naphthalenesulfonic acid.
AE Conjugate (RD)	Anti-AFP antibodies linked to acridinium ester with protein stabilizers.
Calibrator-1 (C0)	High concentration of AFP in Human Serum containing preservatives.
Calibrator-2 (C1)	Low concentration of AFP in Human Serum containing preservatives.
Control-1 (Q1)	Low concentration of AFP with preservatives.
Control-2 (Q2)	High concentration of AFP with preservatives.
Reagent Plugs	Silicon caps to cover the opened reagents.

7. STORAGE AND STABILITY

The shelf-life of the kit is 12 months from the date of manufacturing, when stored at 2-8ºC. **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 0.05% ProClin 300.
- **Assay Cup**
- **Sample Diluent (optional)**
- **J. Mitra’s CLIA Analyzer**

All materials and analyzer to be used for running the AFP i*Clia 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 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

AFP 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.

(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 121°C 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.
9. Mark the test speciment with patient's name or identification number. Improper identification may lead to wrong result reporting.

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 2-8°C.
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 microspheres 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 plug 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 AFP 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 AFP 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 15 μl.
7. Ensure sample positons are properly define at the time of loading in the analyzer.
8. The AFP 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 AFP 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. Every AFP iClia kit has a two-dimension code label containing the predefined master curve of the particular reagent lot.
2. 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 AFP iClia QC data sheet.
3. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless:
4. 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 AFP iClia.

RESULT CALCULATION:

The analyzer calculates cut off values based on the RLUs of calibrator and the results are calculate automatically and given in ng/ml.

Interpretation of Test Results

If sample concentration is lower than the lower limit of the linear range, report the result <0.50 IU/mL, while > 1000.00 IU/mL when it is higher than the upper limit of linear range.

14. DETERMINATION OF REFERENCE INTERVAL

Reference Intervalof this assay is considered as <5.80 IU/mL for healthy people, which is established referring to literatures, based on the rest results of more than 60 clinical samples.

Due to the differences in geography, race, gender or age, it is suggested 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 instruction for use.

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 >10 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 < 0.40 IU/mL

Accuracy: The accuracy of AFP iClia was detected with 30 clinical specimen and compared with Roche CLIA. The co-relation co-efficient is ≥0.990.

Precision

Intra Assay Variation

Within run variation was determined by 5 replicate measurements of two different AFP control sera (Low) and (High) in one assay in 3 different lots. The within assay variability is <15%.

Inter Assay Variation

Between run variation was determined by 05 replicate measurements in 05 sequential days of two different control sera (Low) and (High) in 3 different lots.The between assay variability is <15%.

Intra-Assay, n=5			Inter-Assay, n=5×3		
Control	Mean (IU/ml)	CV	Sample	Mean (IU/ml)	CV
1	9.88	6.18%	1	10.01	10.06%
2	98.7	5.01%	2	98.87	9.67%

Inter machine (CLIA-181 Analyzer) Variation

Between machine variation was determined by 3 replicate measurements of two different AFP control sera (Low) and (High) in 3 different lots in 3 different CLIA-181 Analyzer. The between machine variability is <10%.

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 AFP iClia kit has been demonstrated to be linear from 0.05 IU/ml. to 1000.0 IU/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 AFP concentrations were affected when using the AFP iClia (Thyroid Stimulating Hormone) assay kit. Samples containing the potential interferents were prepared at two AFP concentrations. The samples were assayed, and the AFPconcentrations 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%
Total protein	10 g/dL	<10%
ANA	400AU/mL	< 10%
HAMA	600ng/mL	< 10%

16. LIMITATION OF THE TEST

- The effectiveness of this kit is only confirmed for human serum/plasma, the applicability of other kinds of samples is not verified.
- Clinical diagnosis should not be made on the findings of a single test result, but should be integrate with all clinical and laboratory findings.
- Hemoglobin ≤ 150 mg/dL, triglyceride ≤ 1000 mg/dL, or bilirubin ≤ 40 mg/dL will have no significant interference for the results.

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-manual, 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	Use controls/ calibrator within 30 days once opened and Check storage after expiry. temp. It should be 2-8°C.
	b) Cross contamination	Pipette carefully and do not of Controls interchange caps.