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19. 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 2-8^{0}C.
	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 2-8^{0}C.
	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.
2) False Positive 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 microparticles by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.
3) False negative 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

VER-01 R-00

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MAN/CCL/139
Rev. Date: Mar.-25

CELIAC *i*Clia

Chemiluminesence Immunoassay for the qualitative detection of anti tTG IgA antibodies in Human Serum or Plasma

1. INTRODUCTION

Celiac disease (CD) is an auto immune disease which occurs in predisposed individuals as a reaction to glutein sensitivity. After absorption in the lamina propria of the intestinal mucosa gliadin is deamidated by the tissue transglutaminase (tTG). Gluten is found in various cereals (Wheat, Barley, Rye). If patient with celiac disease consume food containing gluten, this will finally leads to damage to the mucous membranes of the small intestine. The corresponding IgA antibodies are closely corelated to the damage of the small intestine. The test is simple and can be rapidly performed and permit a qualitative assessment of concentration of the transglutaminase antibodies. Although the disease start as in tolerance to gliadins, antibodies to tissue transglutaminase (tTG) in the gut epithelium are characteristic of the disease whereas serum IgA against (tTG) are highly specific for celiac disease, antibodies to gliadin are less informative as they can also be detected in other enteropathy and even in healthy individuals.

2. INTENDED USE

Celiac iCLIA is a is a chemiluminiscent microparticle immunoassay designed for *in vitro* qualitative detection of anti tTG IgA antibodies in Human Serum or Plasma . The assay is intended to be used as an aid in the recognition and diagnosis of Celiac disease. This kit is only operational in connection with J.Mitra CLIA Analyzer.

3. PRINCIPLE

Celiac iClia is chemiluminescent immunoassay based on the “Indirect”principle. The magnetic microparticles are coated with immunodominant epitopes of Recombinant Activated tissue transglutaminase (tTG). The samples /Controls ./Calibrator are added in the assay cup containing coated microparticles. Anti-transglutaminase antibodies if present in the specimen, will bind to the specific tTG antigen absorbed onto the surface of the microparticles. Unbound antibodies are then washed off with wash buffer followed by addition of AE conjugate (Anti-human IgA Antibody linked to acridinium ester) to assay cup. This conjugate will bind to tTg antigen-antibody complex present. The amount of bound AE conjugate is proportional to the concentration of Celiac IgA Antibodies present in the sample. Finally pre-trigger and trigger solution containing hydrogen peroxide and sodium hydroxide solution is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative Light units (RLUs).There is a direct relationship between the amount of Celiac Antibodies (tTG IgA)present in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the Calibrator .

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 microparticles coated with activated tTG recombinant proteins with preservatives.
Sample Diluent (RB)	Buffer containing protein stabilizers & antimicrobial agents as preservative.
Diluent (RC)	Buffer containing protein stabilizers & antimicrobial agents as preservative.
AE Conjugate (RD)	Anti-human IgA linked to acridinium ester with protein stabilizers.
Control-1 (Q1)	Normal human serum, negative for Celiac Antibodies with preservative.
Control-2 (Q2)	Positive for Celiac IgA Antibodies with preservative.
Calibrator-1 (C0)	Cut-off Calibrator, Negative for Celiac Antibodies with preservative.
Calibrator-2 (C1)	Cut-off Calibrator, Positive for Celiac IgA Antibodies with preservative.
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 2-8^{0}C. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8^{0}C.**

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 Celiac 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 2-8^{0}C for one week, or frozen at -20^{0}C 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 viscous/ 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

Celiac 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^{0}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.

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^{0}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 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 plug 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 plug) 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 Celiac iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microparticles buffer that may have settled during transporation/ storage. Once the microparticles 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), (RC) and (RD) bottles and place the reagent plugs before use as follow:

   (RA) & (RB) : Natural color plug
   (RC) : Purple color plug
   (RD) : Brown color plug
3. Load the Celiac 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. Ensure sample positons are properly define at the time of loading in the analyzer.
7. Sample volume required for each additional test from same sample tube is 20 μL. Sample is diluted 1:20 in sample diluent.
8. Sample volume required for each additional test from same sample tube is 20 μl.
9. The Celiac 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.
10. Mix Celiac iClia calibrator and controls by gentle inversion before use. Open the cap and place the calibrator and control-1 & control-2 vials into each respective assigned positions. Read the barcode for calibrator and controls provided with the kit.
11. Run calibration as mentioned in heading **calibration** below.
12. Press Run. The test result for first sample will be obtained at 30 minutes.
13. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Calibration

1. Test Calibrators in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that calibrator and controls values are within the validity range specified in this instruction manual.
2. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless, recalibration is required.
3. 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 and/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 Celiac iClia.

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

i) Sample to cut-off ratio (S/CO) of calibrator-1 (CO) must be between 0.01 to 0.50. If it is not so, the run is invalid and must be repeated or calibrated.

ii) Sample to cut-off ratio (S/CO) of calibrator-2 (C1) must be $\geq$ 0.8. If it is not so, the run is invalid and must be repeated or calibrated.

iii) Sample to cut-off ratio (S/CO) of control-1 (Q1) must be between 0.01 to 0.050. If it is not so, the run is invalid and must be repeated or calibrated.

iv) Sample to cut-off ratio (S/CO) of control-2 (Q2) must be between 5.0 to 20.0. If it is not so, the run is invalid and must be repeated or calibrated.

Note: If one of the Calibrator individual values differ from other 2 replicates, then analyser automatically disregard that value and calculate the calibrator value with the two remaining calibrator values and provide the result.

RESULT CALCULATION:

The analyzer automatically calculates the sample to cut-off ratio (S/CO) of each sample based on cut-off value using formulas.

a. Cut off value = Mean RLU of calibrator-1 + Mean RLU of calibrator-2 x calibration factor (F)

b. Calculation of Sample to cut-off Ratio:

Sample cut-off Ratio (S/CO) = RLU of Sample / Cut-off value

Note: Calibration Factor (F) is batch specific and is provided in the calibrator barcode.

14. INTERPRETATION OF RESULTS

a. If the Celiac S/CO is < 0.9 then interpret the sample as Negative for Celiac antibodies.

b. If the Celiac S/CO is between 0.9 - 1.1 then interpret the sample as Equivocal for Celiac antibodies and sample should be re-tested.

c. If the Celiac S/CO is > 1.1 then interpret the sample as Positive for Celiac antibodies.

15. PERFORMANCE CHARACTERISTICS

A) In-house Evaluation:

Diagnostic Sensitivity and Specificity: The Performance of the Celiac iCLIA with reference to sensitivity and specificity was evaluated in-house with the panel of 79 negative and 10 Celiac positive samples. The performance is also checked with fresh clinical negative (101) and Celiac clinical Positive (12) samples.
The results of all the positive and negative samples were compared with commercially available licensed test kit. The results of the in-house study done are as follows:

No. of Samples	Status	Celiac iClia		Commercially available Celiac ELISA	
		Positive	Negative	Positive	Negative
22	Celiac Positive	22	0	22	0
180	Celiac Negative	0	180	0	180

Sensitivity : 100% **Specificity** : 100%

B) Analytical Specificity :

The analytical specificity of the Celiac iClia Test kit is checked to check the potential for false results with 10 cross-reacting specimen; HIV, HBsAg, HCV, RA and CRP. The specificity on all above samples tested is 100%. The analytical specificity of the test kit is also checked with potentially interfering substances /samples card to check the potential for false results arising from interference from potentially interfering substance .There was no interference with the test results when biomolecules; Bilirubin (20mg/dl), Hemoglobin (500mg/dl),Triglyceride (1000mg/dl), Total protein (10mg/dl), RF (1000mg/ml), ANA (400Au/ml) & HAMA positive human plasma (600ng/mL) were added to the test specimen with much higher level in normal human blood.

Precision: Precision is checked by running Celiac iClia test in 10 replicates (Intra assay variation, Inter assay variation) and Inter Machine variation with Kit controls(Control 1& Control 2), 2 Celiac positive samples; one strong positive and one weak positive. The CV% in Sample RLU to Cutoff ratio (S/CO) of both the controls and positive samples is within 15%.

16. LIMITATION OF THE TEST

1. The Celiac Microlisa is for *in vitro* diagnostic use only.
2. The test should be used for the detection of celiac Antibody in serum or plasma only and not in other body fluids.
3. **This is only a Screening test.** All positive samples must be confirmed by doing intestinal biopsy. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data, should be considered. The results should be reported only after complying with above procedure.
4. Additional follow up testing using available clinical methods (along with repeat Celiac Microlisa test) is required, if Celiac Microlisa test is negative with persisting clinical symptoms.

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. REFERENCES

1. Carroccio A, Lacono G, Montalto G, et. al. Immunology and absorptive tests in celiac disease: Seand J Gasteroenterol 28 (1993).
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