DIAGNOS *CELIAC CARD*

Rapid Visual and sensitive one step test for the Qualitative Detection of Celiac disease associated IgM, IgG & IgA antibodies in Human Serum/Plasma

INTRODUCTION

Celiac disease (CD) is an enteropathy caused by intake of gluten proteins present in cereals such as wheat, barley and rye in genetically predisposed individuals.
Circulating IgA class anti tTG (tissue transglutaminase) and EMA (endomysium) are highly prevalent and characteristic in active celiac disease. Approximately 1% of the population develop celiac disease associated damage of gut epithelium referring to celiac disease at varying ages, but even the majority of the cases may remain undiagnosed despite the development of chronic disease (CD) associated consequences CD can be silent, and such a condition may already lead to unfavourable sequelae.

INTENDED USE

DIAGNOS CELIAC CARD is a visual, rapid, sensitive and accurate one step immunoassay for the qualitative detection of anti tTG IgA, IgG & IgM antibodies in Human Serum or Plasma. The assay is intended to be used as an aid in the recognition and diagnosis of Celiac disease.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

DIAGNOS CELIAC CARD is a one step immunoassay based on the antigen capture, or "sandwich" principle. The method uses tissue transglutaminase (tTG) antigen conjugated to colloidal gold and the same antigen is immobilized on a nitrocellulose strip. The diluted test sample is introduced to and flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains celiac disease specific antibody, the colloidal gold-antigen conjugate binds to the antibody, forming an antibody-antigen-colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized tTG antigen (Test line) "T", the complex is trapped forming an antigen-antibody-antigen colloidal gold complex. This forms a pink band indicating the sample is reactive for celiac disease specific antibody. Absence of a colored band in the test region indicates a non-reactive test result. A red procedural control line should always develop at 'C' region to indicate that the test has been performed properly.

MATERIALS PROVIDED

DIAGNOS CELIAC CARD Test kit contains following components to perform the assay:

1. Diagnos Celiac Card	2. Assay Buffer	3. Sample Dropper
4. Transfer Dropper	5. Sample Cup	6. Instruction Manual

KIT PRESENTATION

10 Test Pack

KIT STORAGE & STABILITY

Store all components at 2-8ºC when not in use. Expiry date on the kit indicates the date beyond which the kit should not be used. The DIAGNOS CELIAC CARD should not be frozen and must be protected from exposure to humidity.

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 British and European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		See 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
	Single use only		Keep away from sunlight
	Do not use if package is damaged		

WARNING FOR USERS

CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT 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 disposed off in accordance with established safety procedures and guidelines.
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. Do not open the foil pouch to remove the product until it attains room temperature and you are ready to perform the test.
10. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.
11. Optimal test performance requires strict adherence to the test procedure described in the insert.
12. Assay Buffer contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).

PRECAUTIONS

In order to obtain reproducible results, the following instructions must be followed:

1. Do not mix reagents from different batches.
2. Do not re-use the test device.
3. Use separate sample dropper or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
4. Follow the given test procedure and storage instructions strictly.

SAMPLE / SPECIMEN COLLECTION & STORAGE

a) DIAGNOS CELIAC CARD should be performed on human serum or plasma only immediately after collection.

b) If not tested immediately, specimen should be refrigerated at 2-8ºC upto 3 days following collection.

c) If testing within 3 days is not possible, specimen should be stored frozen at -20ºC.

d) Specimen containing visible precipitates or cloudy specimens may give inconsistent test results. Such specimens should be clarified prior to testing by high speed centrifugation i.e. 10,000 rpm for 15 minutes before testing.

e) Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

TEST PROCEDURE

1. Bring the required number of DIAGNOS CELIAC CARD foil pouches, Assay Buffer vial and specimen to room temperature prior to testing. **RT 20-30°C**
2. Remove the test card from the foil pouch prior to use.
3. Label the test card with patient's name or identification number.
4. Add 5 drops of Assay Buffer to the Sample Cup as shown in fig. (a)
5. Add 5µl sample using Sample Dropper in Sample Cup containing Assay Buffer as shown in fig. (b). Mix properly & make sure bubbles are not formed while mixing.

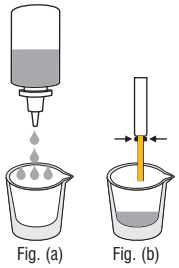

Fig. (a) Fig. (b)

OR

(Alternatively, using a micropipette take 5μl sample.)

6. Using the Transfer Dropper provided, transfer 3 drops of this mixture from Sample Cup into sample well of DIAGNOS CELIAC CARD as shown in fig.(c).
7. Dispose off the Sample Dropper, Transfer Dropper and Sample Cup containing remianing mixure of the diluted sample considering it biohazardous .

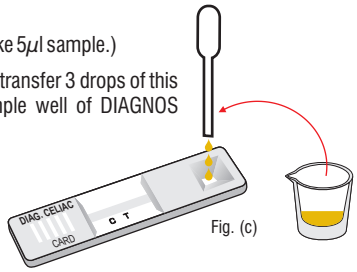

Fig. (c)

8. Allow reaction to occur during next 20 minutes.
9. Read results in 20 minutes. Positive results may appear as early as 2-10 minutes. However negative results must be confirmed at the end of 20 minutes only.
10. Discard the DIAGNOS CELIAC CARD immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF RESULT

REACTIVE :

Fig. (d)

As shown in Fig. (d), appearance of pink coloured line, one each in test region "T" and control region "C" indicates that the sample is REACTIVE for celiac disease specific antibody. A difference of intensity in colour may occur between the Test line & Control line depending on the concentration of the celiac disease specific antibody in the sample but this does not affect interpretation of the result.

NON-REACTIVE :

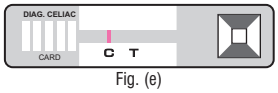

Fig. (e)

As shown in Fig. (e) appearance of one distinct pink line in the control region "C" only, indicates that the sample is "NON REACTIVE" for celiac disease specific antibody.

INVALID :

The test is invalid if no control line appears after completion of test as shown in Fig. (f) & (g). Invalid results may be because of the following reasons:

Fig. (f)

Fig. (g)

a) Improper storage at temperature other than the recommended temperature.
b) Wrong procedure.
c) Long atmospheric exposure of the test device after opening the pouch.

The test should be repeated using a new DIAGNOS CELIAC CARD and test sample.

LIMITATIONS OF THE PROCEDURE

1. The DIAGNOS CELIAC CARD is for in vitro diagnostic use only.
2. The test should be used for the detection of celiac IgM, IgG & IgA Antibody in serum or plasma only and not in other body fluids.
3. This is only a Screening test. All reactive samples should be confirmed by confirmatory test. 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 DIAGNOS CELIAC CARD test) is required, if DIAGNOS CELIAC CARD test is non-reactive with persisting clinical symptoms.
5. False positive results can be obtained due to the presence of other immune complexes or other immunoglobulin aggregates in the patients sample. This occurs in less than 1% of the samples tested.

PERFORMANCE CHARACTERISTICS

The Diagnos Celiac Card performance with regard to Sensitivity and Specificity has been carried out in-house on fresh as well as frozen samples from low as well as high risk group as follows:.

IN-HOUSE EVALUATION

The clinical sensitivity of the DIAGNOS CELIAC CARD was determined using 273 positive sera from patients with celiac disease having $\geq$200 RU/ml (samples taken from Pediatric Deptt., AIIMS, New Delhi) and the specificity was checked with a panel of 2000 plasma and serum sample which were ELISA negative for Celiac antibodies. The results obtained are as follows:

SENSITIVITY : 99.26% **SPECIFICITY : 99.50%**

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of 6 specimens: one negative and five celiac antibody positive; two weak and two moderate and one strong positive. The C.V (%) of negative, weak positive, moderate positive and strong positive samples were within 10% of the time.

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 manufacturer'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.

BIBLIOGRAPHY OF SUGGESTED READING

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For in-vitro diagnostic use only, not for medicinal use

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