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Instruction For Use
2012-11

CE

US Market: For Research Use Only

ORG 740 Gastro-5-Line

NAME AND INTENDED USE

Gastro-5-Line Immunoblot assay is a membrane based enzyme immunoassay for the semi-quantitative measurement of IgG and IgA class autoantibodies to intrinsic factor, parietal cell H+/K+-ATPase, tissue transglutaminase, Mannan from Saccharomyces cerevisiae and gliadin in human serum or plasma. The assay is intended for research use only.

SYMBOLS USED

IVD	In vitro diagnostic medical device	BLOT STRIPS	Blot strips
	Manufacturer	DILUENT	Sample Buffer
REF	Catalogue number	CONJUGATE	Enzyme Conjugate
	Sufficient for	WASH	Wash Buffer
LOT	Batch code	BCIP	BCIP Substrate
	Use by	RTU	Ready to use
	Temperature limitation		
	Consult instructions for use		
	Keep away from sunlight		
	Do not reuse		
	Date of manufacture		

SUMMARY AND EXPLANATION OF THE TEST

Every section of the gastrointestinal tract is prone to its own unique disorders. Crohn's disease, celiac disease, ulcerative colitis and pernicious anaemia are four of the more serious diagnoses that imply a long-term tendency to digestive tract diseases. These digestive tract diseases are immune-mediated, with increased permeability of the digestive tract, and are associated with a long list of whole body immune-mediated diseases. Patients with these conditions are often symptomatic for many years before the diagnosis is made.

Intrinsic Factor

Serum intrinsic factor autoantibodies can be detected in 50 to 70% of pernicious anaemia patients and are highly specific for Biermer´s anaemia with no reported single true positive in a healthy control [1]. Two types of intrinsic factor autoantibodies exist [2]. Type I antibodies block the cobalamin binding site on the intrinsic factor molecule, preventing uptake of the vitamin. Type II antibodies block a different site of the intrinsic factor molecule that is involved in binding of the intrinsic-factor-cobalamin-complex to ileal receptors. Both types of antibodies have the same pathological effect, i.e. preventing cobalamin resorption by ileal receptors.

Parietal Cell

Circulating autoantibodies to gastric parietal cells can be detected in 80-90% of pernicious anemia patients and they are also detected in 2-5% of the healthy adult population [3]. Biochemical and molecular investigations identified the responsible antigens as alpha- and beta-subunit of the gastric H+/K+ ATPase. The gastric H+/K+ ATPase (EC 3.6.1.3) is a hydrogen transporting enzyme, responsible for the acidification of the stomach lumen [4].

Tissue Transglutaminase

Anti-tTG IgA is a highly sensitive marker for celiac disease with 95-100 %, and have a specificity of 90 to 97 %.The enzyme tissue Transglutaminase (tTG) has been reported to be the main, if not sole, target for endomysial antibodies. These antibodies fall once a gluten-free diet has begun, thus facilitating monitoring of dietary compliance [5, 6, 7].

ASCA

ASCA are directed against oligo-mannosidic epitopes on the cell wall mannan (phosphopeptido-mannan) of the yeast Saccharomyces cerevisiae [8]. To differentiate between Crohn´s disease and ulcerative colitis the detection of ANCA (anti neutrophil cytoplasmic antibody) and ASCA (anti-Saccharomyces cerevisiae antibody) can be used. IgG as well as IgA ASCA shows a specificity of 95-100% for Crohn´s disease. ASCA are strongly associated to Crohn´s disease. Studies showed 5% positive IgG and 7% IgA class ASCA in ulcerative colitis whereas in Crohn´s disease a sensitivity of 75% for IgG and 60% for IgA class ASCA could be observed [9, 10].

Gliadin

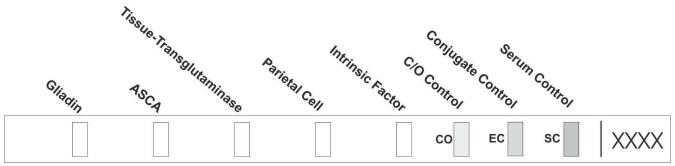
Celiac patients exposed to gluten express high levels of serum antibodies to gliadin and tTG. Elimination of gluten from their diet results in a decrease of the titer of these antibodies and to their eventual disappearance. Gliadins are proteins containing high amounts of the amino acids proline and glutamine. This protein belongs to the nutritive tissue of the grain seeds of wheat, oat, barley and rye. The increased association of celiac disease with selective IgA deficiency is a potential source of false-negative IgA. Therefore testing for IgG class autoantibodies is recommended if celiac disease is suspected [11, 12].

The Gastro-5-Line combines the advantages of immunoblot technique with a set of well selected antigens.

PRINCIPLE OF THE TEST

Highly purified antigens intrinsic factor, parietal cell H+/K+-ATPase, tissue transglutaminase, Mannan from *Saccharomyces cerevisiae* and gliadin as well as three control antigens for CO Cut-off Control, EC Enzyme Conjugate Control and SC Serum Control are bound to nitrocellulose membrane blot strips.

Autoantibodies present in serum or plasma bind to the immoblizied antigen. Washing of the blot strips removes unbound antibodies and unspecific sample components. Alkaline phosphatase conjugated anti-human IgG and IgA detect the bound sample antibodies forming a conjugate/antibody/antigen complex. Washing of the blot strips removes unbound conjugate. The substrate BCIP/NBT is hydrolized by bound enzyme conjugate to form an insoluble blue-violet product. Washing of the blot strips removes unhydrolyzed substrate and stopps the reaction. The amount of color is directly proportional to the concentration of IgG and IgA antibodies present in the original sample.



WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for research use only.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate BCIP/NBT.
- Sample buffer and wash buffer contain sodium azid 0.09% as preservative. This concentration is classified non-hazardous.
- Enzyme conjugate contains 0.05% ProClin as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store subtrate solution in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
- Avoid repetitive freezing and thawing of serum samples.
- Testing of heat-inactivated sera is not recommended.

CONTENTS OF THE KIT

▽16	ORG 740-16	Sufficient for 16 determinations
▽8	ORG 740-08	Sufficient for 8 determinations
BLOT STRIPS	▽16	8 antigen coated nitrocellulose strips. Ready to use. 1 pre-developed calibration strip (coded CAL) for semiquantitative evaluation. Ready to use. Product code on strip: ***740*** Code on Calibration strip: ***CAL***
DILUENT	1x 20 ml	Sample Buffer PG, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow. Ready to use.
CONJUGATE	1x 15 ml	Enzyme Conjugate containing anti-human IgG and IgA antibodies, alkaline phosphatase labelled; PBS, BSA, detergent, preservative ProClin 0.05%, light red. Ready to use.
WASH	1x 20 ml	Wash Buffer WB, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
BCIP	1x/2x 13 ml	BCIP Substrate; containing BCIP/NBT. Ready to use.
	1x/2x	Incubation tray
	1x	Instruction for Use: ELISA Mini-CD0
	1x	Certificate of Analysis

MATERIALS REQUIRED

- Pipettes for 10 µl and 1000 µl
- Distilled or deionised water
- Graduated cylinder for 1000 ml
- Laboratory timing device
- Rocking platform
- Tweezers

STORAGE AND STABILITY

- Store the kit at 2-8 °C.
- Keep nitrocellulose strips carefully sealed in the original plastic tube with desiccants provided.
- Important: The calibration strip is very light-sensitive. Store in the dark!
- Do not expose test reagents to heat, sun or strong light during storage and usage.
- The unopened test kit is stable for 18 months from day of production. See expiry date on outer labels for individual batches.
- Diluted wash buffer is stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots.
- All materials must be at room temperature (20-28 °C).
- Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions
- To avoid carryover contamination, change the tip between samples.
- All incubation steps must be accurately timed.
- Control sera should routinely be assayed as unknowns to check performance of the reagents and the assay.
- Nitrocellulose strips must be handled with gloves or tweezers.
- It is important to make sure, that air-bubbles do not interfere with the strip during incubation. This could cause irregularities in coloration of developing bands and can lead to wrong results.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Ready to use.

Preparation of samples

Sample dilution see test procedure. Effective dilution during test is 1:101.

TEST PROCEDURE

Using tweezers insert one nitrocellulose strip into one chamber of the incubation tray:

- Add **1.0 ml sample buffer** to the strip in the chamber.
- Allow to equilibrate for 5 minutes with gentle bobbing.
- Add **10 µl of patient sample** directly to the chamber.
- Incubate for **60 minutes** at room temperature (20-28 °C) with gentle bobbing.
- Remove the diluted sample completely from the chamber.
- Add 2.0 ml wash buffer to the chamber, incubate for 5 minutes.
- Remove wash buffer completely. Repeat this procedure twice.

- Add **1.0 ml enzyme conjugate** to each strip in the chamber of the incubation tray.
- Incubate for **30 minutes** at room temperature with gentle bobbing.
- Remove the conjugate completely from the chamber.
- Add 2.0 ml wash buffer to the chamber, incubate for 5 minutes.
- Remove wash buffer completely. Repeat this procedure twice.

- Add **1.0 ml substrate** to each strip in the chamber of the incubation tray.
- Incubate for **10 minutes** at room temperature with gentle bobbing.
- Remove the substrate completly.
- Add 1.0 ml distilled water to the chamber, incubate for 5 minutes.
- Remove water completely. Repeat this procedure twice.

Carefully blot the strips with a tissue paper. Allow strips to air dry before evaluating with the calibration strip.

VALIDATION

The assay is valid if the all three control lines (**CO** Cut-off Control, **EC** Enzyme Conjugate Control and **SC** Serum Control) show a turn-over of substrate in terms of blue-violet lines! If this criteria is not met the assay is invalid and should be repeated.

Note: Borderline samples should be repeated or tested using an alternative procedure. Samples from patients diagnosed with autoimmune diseases often show multiple autoantibody specificities. Such samples may show a positive reaction with more than one antigen line.

CALCULATION OF RESULTS

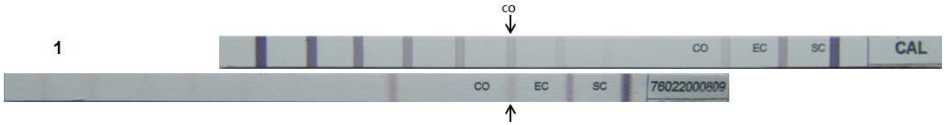
The intensity of a **blue-violet line** at the position of the coated antigen is directly proportional to the concentration of IgG and IgA antibodies present in the sample tested.

Semi-quantitative evaluation of sample strip:

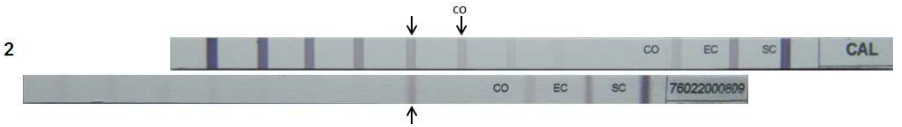
negativ	intensity of patient sample line weaker than intensity of CO-line
borderline	intensity of patient sample line eqivalent to intensity of CO-line
weak positive	intensity of patient sample line up to 1 level stronger than intensity of CO-line
positive	intensity of patient sample line up to 2 levels stronger than intensity of CO-line
strong positive	intensity of patient sample line ≥3 levels stronger than intensity of CO-line

Interpretation of the intensity of blue-violet lines:

(1) Compare intensity of the **CO-line of the sample strip** to the intensity of the lines of the calibration strip.
Example:



(2) Compare the intensity of the **patient sample line** to the intensity of the lines of the calibration strip.
Example: Interpretation of intensity of patient sample line is "weak positive".



PERFORMANCE CHARACTERISTICS

CALIBRATION

The sensitivity, specificity and dose response of the Gastro-5-Line immunoblot was evaluated using clinically defined in house quality control sera containing varying relative amounts of sera with known specificity.

Measuring range

The evaluation of the intensity of the blue lines as described above allows a semi-quantitative determination of IgG and IgA class autoantibodies in the sample tested into quantification ranges:

negative, borderline, weak positive, positive, strong positive

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this assay. Cut-off: borderline

Interpretation of results

normal:	negative
elevated:	weak positive, positive, strong positive

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity of each dilution step was determined using the calibration strip.

Linearity				
Sample	Dilution	Observed	Expected	O/E
1	1:100	strong positive	strong positive	PASS
.	1:200	positive	positive	PASS
.	1:400	weak positive	weak positive	PASS
.	1:800	borderline	borderline	PASS
.	1:1600	negative	negative	PASS
2	1:100	strong positive	strong positive	PASS
.	1:200	positive	positive	PASS
.	1:400	weak positive	weak positive	PASS
.	1:800	borderline	borderline	PASS
.	1:1600	negative	negative	PASS

Sensitivity

This immunoblot assay is a semi-quantitative assay method. Any reactivity less than borderline is considered negative and cannot be quantified any further.

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean	Result
1	negative	PASS
2	weak	PASS
3	positive	PASS

Inter-Assay		
Sample	Mean	Result
1	negative	PASS
2	weak	PASS
3	positive	PASS

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n pos	%
Coeliac disease	100	98	98.0
Pernicious anaemia	85	74	87.1
Normal human sera	150	6	4.0

			Clinical Diagnosis		
			Pos	Neg	
ORG 740	Pos		172	6	
Gastro-5-Line	Neg		13	144	
Sensitivity:	93.0		185	150	335
Specificity:	96.0	%			
Overall agreement:	94.3	%			
		%			

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

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1. Add **blot strip** into the incubation tray
   → Add **1000 µl** sample buffer per strip into the incubation tray
   → Shake **5 minutes** while incubating

2. Add **10 µl** patient sample and resuspend
   → Shake **60 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **2000 µl** wash buffer, discard wash

3. Add **1000 µl** enzyme conjugate solution per strip
   → Shake **30 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **2000 µl** wash buffer, discard wash

4. Add **1000 µl** substrate per strip
   → Shake **10 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **1000 µl distilled water**, dry blot strips. Read after complete drying, only

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