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Instruction for use  
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## ORG 740-08 / ORG 740-16 Gastro-5-Line

Membrane-based immunoblot for the determination of autoantibodies against Intrinsic Factor, Parietal Cell, tissue Transglutaminase, *Saccharomyces cerevisiae* (ASCA) and Gliadin

For research use only. Not for use in diagnostic procedures.

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### NAME AND INTENDED USE

Gastro-5-Line is a membrane based enzyme immunoassay for the determination of IgG and IgA class autoantibodies to Intrinsic Factor, Parietal Cell, tissue Transglutaminase, *Saccharomyces cerevisiae* (ASCA) and Gliadin. The assay is intended for research use only, not for use in diagnostic procedures.

### PRINCIPLE OF THE TEST

Highly purified antigens are bound to nitrocellulose membrane strips. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the membrane strips removes unspecific serum and plasma components. Alkaline phosphatase conjugated anti-human IgG and IgA immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the membrane strips removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyses to form an insoluble blue-violet product. Washing of the membrane strips removes unhydrolysed substrate. The amount of colour is directly proportional to the concentration of IgG and IgA antibodies present in the original sample.

### WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for research use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the substrate solution BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/p-nitro blue tetrazolium chloride). If BCIP/NBT comes into contact with skin, wash thoroughly with water and soap.
5. Some kit components (i.e. controls, sample buffer and buffered wash solution) contain sodium azide as preservative. Sodium azide ( $\text{NaN}_3$ ) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 7., 8., 9.)..
6. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in laboratory areas.

Observe the guidelines for performing quality control by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

## CONTENTS OF THE KIT

Package size	8 or 16 determ.
Qty. 8 or 16	Nitrocellulose strips, loaded with highly purified native or recombinant antigens. Ready to use..
1 vial, 20 ml	Sample buffer. Ready to use. This buffer is specifically adapted for the Gastro-5-Line and is not interchangeable with sample buffers of other immunoblots.
1 vial, 20 ml	Wash buffer, concentrate (50x).
1 vial, 20 ml	Enzyme conjugate solution (PBS, $\text{NaN}_3$ <0.1 % (w/w)), (pink) containing polyclonal rabbit anti-human-IgG and polyclonal rabbit anti-human-IgA; labelled with alkaline phosphatase. Ready to use.
1 or 2 vials, 10 ml	Substrate solution (BCIP/NBT). Ready to use.
Qty. 1 or 2	Pre-developed nitrocellulose calibration strip (labelled CAL) for semi-quantitative evaluation. Ready to use.
Qty. 1 or 2	Incubation tray.
Qty. 1 or 2	Documentation sheet. Ready to use.

## STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep nitrocellulose strips dry; store together with desiccant and carefully sealed in the plastic tube.
3. **Important:** The calibration strip is very light-sensitive. Please store dark.
4. The reagents are stable until expiration of the kit.
5. Do not expose test reagents to heat, sun or strong light during storage and usage.
6. Diluted sample buffer and wash buffer are stable for at least 30 days if stored at 2-8 °C.

## MATERIALS REQUIRED

### Equipment

- Pipettes for 10  $\mu\text{l}$  and 1000  $\mu\text{l}$
- Laboratory timing device
- Rocking platform
- Tweezers

### Preparation of reagents

- Distilled or deionised water
- Graduated cylinder for 1000 ml

## COLLECTION, STORAGE AND HANDLING OF UNKNOWNNS

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

## PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. 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.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. To avoid carryover contamination, change the tip between samples and different kit controls.
8. Nitrocellulose strips must be handled with gloves or tweezers.
9. All incubation steps must be accurately timed.
10. Control sera or pools should routinely be assayed as unknownns to check performance of the reagents and the assay.
11. 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

### Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

**TEST PROCEDURE**

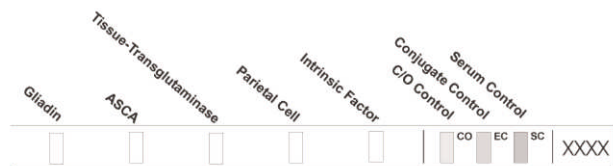
1. Insert a Gastro-5-Line strip using tweezers then add 1.0 ml sample buffer to each chamber of the incubation tray. Allow to equilibrate for 5 minutes with gentle rocking.
2. Add 10 µl of sample serum directly to the chamber (effective dilution 1:101).
3. Incubate for 60 minutes at room temperature (20-28 °C).
4. Carefully remove the diluted serum completely from the strips.
5. Add 2.0 ml wash buffer, incubate for 5 minutes, and then remove as in step 4. Repeat this procedure twice.
6. Add 1.0 ml enzyme conjugate to each chamber.
7. Incubate for 30 minutes with gentle rocking at room temperature.
8. Remove the diluted conjugate completely from the strips.
9. Add 2.0 ml wash buffer, incubate for 5 minutes, and then remove as in step 4. Repeat this procedure twice.
10. Add 1.0 ml substrate to each strip.
11. Incubate for 10 minutes with gentle rocking at room temperature.
12. Remove the substrate and wash the strips with 1 ml distilled water three times 5 minutes each to stop the reaction.
13. Carefully blot the strips dry with a paper towel.
14. Allow strips to air dry before evaluating.

**RESULTS**

**Quality Control**

This test is only valid if the Serum Control (first line), Conjugate Control (second line) and Cut-Off Control (Third line) show a turn-over of substrate in terms of developed bands! If these criteria are not met, the result is invalid and the test should be repeated.

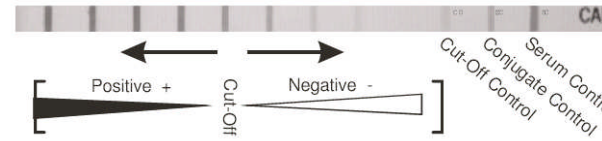
The antigens are coated on the membrane in the order illustrated in the figure below.



The developed lines are compared with the calibration strip as follows:

1. Compare the Cut-Off control line of the sample strip to the calibration lines on the calibration strip to adjust.

2. Compare the unknown lines to the calibration lines adjusted to the Cut-Off control for determination



Notes to results:

1. This assay is for the determination of the specificity of autoantibodies in sample serum, allowing discrimination between negative, borderline, weak positive, positive, and strong positive. Borderline samples should be repeated or tested using an alternative procedure.

**PERFORMANCE CHARACTERISTICS**

**Specificity**

The Gastro-5-Line test was evaluated by testing sera of known specificity and blood donor sera using the general test procedure. All blood donors gave negative lines for all antigen specificities.

**Calibration**

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

**LIMITATIONS OF PROCEDURE**

The Gastro-5-Line immunoblot assay is intended for research use only.

**INTERFERING SUBSTANCES**

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

## INCUBATION SCHEME

- 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