DRG® Anti-Intrinsic Factor (IFAb) (EIA-4392)

Revised 29 June 2010 rm (Vers. 2.1) **RUO in the USA**

NAME AND INTENDED USE

Anti-Intrinsic Factor ELISA is an indirect solid phase enzyme immunoassay (ELISA) for the measurement of IgG class autoantibodies against intrinsic factor in human serum or plasma for the determination of pernicious anemia.

SUMMARY AND EXPLANATION OF THE TEST

Biermer´s anaemia or pernicious anaemia is the most common cause of vitamin B12 deficiency in Western populations showing the classical features of megaloblastic anaemia (i.e. morphologic and functional abnormalities of the blood cells and marrow precursors related to impairment of DNA synthesis) [1, 2, 3]. It is characterised by a gastric mucosal defect that decreases the synthesis of intrinsic factor and the occurence of autoantibodies to gastric parietal cells and to intrinsic factor. Human intrinsic factor is a glycoprotein that is exclusively produced by gastric parietal cells. It plays an essential role in the absorption and transport of vitamin B12 across the small intestine [4].
Two types of intrinsic factor autoantibodies exist [5]. 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.
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 [6].
Anti-Intrinsic Factor ELISA detects both types of autoantibodies and thereby provides a useful tool in the determination of pernicious anaemia and other causes of vitamin B12 malabsorption.

PRINCIPLE OF THE TEST

Human recombinant intrinsic factor is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unbound serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG 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 TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.

6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (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 8., 9., 10.).
7. 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.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories 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	96 determ.
Qty.1	Divisible **microplate** consisting of 12 modules of 8 wells each, coated with human recombinant intrinsic factor.
6 vials, 1.5 ml each	Anti-Intrinsic factor **Standards** (0-5) in a serum/buffer matrix (PBS, NaN_3<0.1% (w/w)) containing: 0; 6.3; 12.5; 25; 50; 100 U/ml. Ready to use.
2 vials, 1.5 ml each	Anti-Intrinsic factor **controls** in a serum/buffer matrix (PBS, NaN_3 <0.1% (w/w)). Positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.
1 vial, 20 ml	**Sample Buffer** (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	**Enzyme Conjugate** solution (PBS, PROCLIN 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**TMB Substrate Solution**. Ready to use.
1 vial, 15 ml	**Stop Solution** (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	**Wash Solution** (PBS, NaN_3 <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

Revised 29 June 2010 rm (Vers. 2.1) **RUO in the USA**

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens 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-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens 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. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer
Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 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.

Preparation of wash solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized 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.

Sample preparation
Serum or plasma can be used for this assay.
Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.
Standards and Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate standards, controls and prediluted patient samples.
2. Pipet 100 µl of standards, controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	S0	S4	P1	P..		
B	S0	S4	P1	P..		
C	S1	S5	P2			
D	S1	S5	P2			
E	S2	C1	P3			
F	S2	C1	P3			
G	S3	C2	P4			
H	S3	C2	P4			

S0 to S5: Standard 0 to 5
P1, P2... patient sample 1, 2 ...
C1: positive control
C2: negtive control

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of *Enzyme Conjugate* into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

8. Dispense 100 µl of *TMB Substrate Solution* into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of *Stop Solution* to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.
    The developed colour is stable for at least 30 minutes. Read optical densities during this time.

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 hemolyzed or lipemic samples should be avoided.

REFERENCES

1. Gleeson, P.A., and B.-H. Toh. Molecular targets in pernicious anaemia. Immun. Today, 1991, 12(7):233-238.
2. Beck, W.S.. Neuropsychatric Consequences of Cobalamin Deficiency. Adv. Inter. Med. 1991, 33-56.
3. OH R, Brown DL. Vitamin B12 deficiency. Am Fam Physician. 2003, 67(5): 979-986.
4. Seetharam, B., S. Bose, and N. Li. Cellular Import of Cobalamin (Vitamin B-12). J.Nutr., 1999, 129:1761-1764.
5. Schade, S.G., P.L. Feick, M.H. Imrie, and R.F. Schilling. In vitro studies on antibodies to intrinsic factor. Clin. Exp. Immunol. 1967, 2:399-413.
6. Carmel, R.. Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race. Clin. Exp. Immunol., 1992, 89:74-77.