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Anti-Intrinsic Factor

ORG 647

96 Tests

Immunometric Enzyme Immunoassay for the quantitative determination of Anti-Intrinsic Factor

CE

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NAME AND INTENDED USE

Anti-Intrinsic Factor is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against intrinsic factor in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of pernicious anaemia.

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].

ORGENTEC Anti-Intrinsic Factor ELISA detects both types of autoantibodies and thereby provides a useful tool in the differential diagnosis of pernicious anaemia and other causes of vitamin B12 malabsorption.

PRINCIPLE OF THE TEST

Human recombinant intrinsic factor is bound to microwells. Antibodies to this antigen, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically bind to 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 in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg and HIV by FDA approved methods. No test can guarantee the absence of HBsAg or HIV, and so all human serumbased 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 contains 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 (NaN3) is highly toxic and reactive in pure form. At the product concentrations, though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 7., 8., 9.)
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 Calibrators (A-F) in a serum/buffer matrix (PBS, NaN3<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, NaN3 <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, NaN3 <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, NaN3 <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

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

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. Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of controls and prediluted patient samples in duplicate into the wells.
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 color is stable for at least 30 minutes. Read optical densities during this time.

Automation

The ORGENTEC Anti-Intrinsic Factor ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not met, the results are invalid and the test should be repeated.

Calculation of results

For Anti-Intrinsic Factor a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Intrinsic factor test:

	Anti-Intrinsic factor IgG
Negative:	< 6 U/ml
Positive:	> 6 U/ml

Positive results should be verified concerning the entire clinical status of the patient. Also every decission for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges. The value above should be regarded as guideline only.

PERFORMANCE CHARACTERISTICS

Precision (Reproducibility)

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 9 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 3 different runs with 24 determinations of each sample:

Intra-Assay		
Sample No.	Mean (Index Value)	CV [%]
1	>200	1.5
2	103	3.2
3	32	2.7

Inter-Assay		
Sample No.	Mean (Index Value)	CV [%]
1		
2		
3		

Sensitivity

The lower detection limit for Anti-Intrinsic factor IgG was determined at 1 U/ml.

Specificity

The solid phase is coated with human recombinant intrinsic factor. Therefore the Anti-Intrinsic factor test kit recognizes only autoantibodies specific for intrinsic factor.

Calibration

Since no international reference preparations for Anti-Intrinsic factor autoantibodies is available, the assay system is calibrated in arbitrary units.

LIMITATIONS OF PROCEDURE

The Anti-Intrinsic factor ELISA is for use only as part of a diagnostic profile for pernicious anemia, the results of which should be evaluated together with other clinical and laboratory evidence, as interpretated by an appropriate physician.

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 be avoided.

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INCUBATION SCHEME

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**