3. Principle of the test

Diluted serum samples are incubated with highly purified (>99%) hog intrinsic factor immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, controls and samples is measured using a microplate reader at 450nm. Optical density is directly proportional to antibody activity in the sample.

4. Materials included in the kit

- Microplate: 96 wells in 12 X 8 break-apart strips, pre-coated with hog intrinsic factor, with holder in a foil bag with desiccant.
- Reagent 1: Sample Diluent 62.5mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 16ml, (blue), concentrate x6.25
- Reagent 2: Wash Buffer 150mM Tris-buffered saline with detergent, pH 7.2, 75 ml, concentrate x15
- Reagent 3: Conjugate rabbit anti-human IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, (red), ready to use
- Reagent 4: TMB Substrate aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- Reagent 5: Stop Solution 0.25M sulphuric acid, 12 ml, ready to use
- Standards: 0 & 10U/ml, 2ml of 10mM Tris-buffered saline containing human serum IgG antibodies to intrinsic factor, ready to use
- Positive Control: 2ml of 10mM Tris-buffered saline containing human serum antibodies to intrinsic factor, ready to use
- Negative Control: 2ml of 10mM Tris-buffered saline containing normal human serum, ready to use

6. Precautions

6.1 Safety Precautions

1. All reagents in this kit are for research use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of standards and controls for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.

6.2 Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. The sample diluent x6.25 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength to last the run. However, if the working strength becomes cloudy, discard and prepare fresh.

Intrinsic Factor IgG ELISA Kit
Qualitative/semi-quantitative assay for Intrinsic Factor IgG antibodies

For Research Use Only. Not For Use In Diagnostic Procedures.
1. Dilute patient samples 1:100 in diluted sample diluent (eg 10 ml of each control and samples). 
2. Positive results should not be used as sole diagnostic criteria for Biermer’s anaemia. Results must be correlated with haematologic findings.
3. Positive results should be interpreted in conjunction with further autoimmune testing as antibodies to intrinsic factor may be associated with other autoimmune diseases. The Gastric Parietal Cell antibodies kit (GD35) may be useful.
4. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette.
5. Include the positive and negative control in every test run to monitor for substantial reagent failure. Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.
6. When pipetting Conjugate or Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
7. Do not allow microwells to dry between incubation steps.
8. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for enzyme conjugate completely separate from the substrate reagent.
9. Blot the wells on absorbent paper before proceeding.
10. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

5. After 30 minutes, decant or aspirate the well contents and wash the wells 4 times with wash buffer. Ensure that the wells are empty but do not allow to dry out.

6. Dispense 100µl of Conjugate (Reagent 3) into each well. Incubate the wells for 30 minutes at room temperature.

7. After 30 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are empty but do not allow to dry out.

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (Reagent 4) into each well. Incubate the plate for 10 minutes.

9. Add 100µl of Stop Solution (Reagent 5) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 520nm filter may be used as a reference wavelength.

11. Quality control

Quality control data is supplied on the lot-specific QC certificate included in the kit. Controls are intended to monitor for substantial reagent failure.

12. Interpretation of Results

Patients with pernicious anaemia have values above 10 U/ml. Ideally, each laboratory should establish its own normal range data.

Qualitative results

Samples with OD > than OD of 10 U/ml standard + 12% are positive.

Samples with OD < than OD of 10 U/ml standard – 12% are negative.

Semi-Quantitative results

Semi-quantitative results can be obtained by the following equation.

Patient result = Sample OD – 0 U/ml OD

10 U/ml standard OD – 0 U/ml OD

Samples falling in the range 8.5 – 11.5 U/ml are indeterminate.

Quantitative results

An optional set of Intrinsic factor standards can be purchased separately to allow quantitative measurement of IF antibodies. If the optional standard set is used, plot the optical density of each standard against its concentration and draw the best-fit curve through the points. Read the unknowns off this curve.

13. Limitations of the Procedure

1. Positive results should not be used as sole diagnostic criteria for Biermer’s anaemia. Results must be correlated with haematologic findings.
2. Positive results should be interpreted in conjunction with further autoimmune testing as antibodies to intrinsic factor may be associated with other autoimmune diseases. The Gastric Parietal Cell antibodies kit (GD35) may be useful.

14. Performance Characteristics

Clinical Studies

Patients with pernicious anaemia and normal donors were assessed using the Intrinsic Factor IgG kit. Based on the results obtained, diagnostic sensitivity and specificity was 95% and 98%, respectively.

15. Reproducibility

Within assay coefficient of variation < 6%

Between assay coefficient of variation < 12%
Method Summary

• Dilute sera 1:100 with sample diluent (Reagent 1)
• Dispense 100 µl standards, the positive and negative controls and the diluted sample into the microplate wells
• Incubate for 30 minutes at room temperature.
• Wash the wells three times
• Dispense 100µl of conjugate (Reagent 3) into each well
• Incubate at room temperature for 30 minutes
• Wash the wells four times
• Add 100µl of TMB substrate (Reagent 4) to each well
• Incubate at room temperature for 10 minutes
• Add 100µl stop solution (Reagent 5) to each well
• Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

Further reading


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ALPCO Diagnostics
PO Box 451 • Windham, NH  03087
Tel (800) 592-5726 • Fax (603) 898-6854
www.alpco.com • email@alpco.com