ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

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Biermer Atrophic Gastritis DOT KIT

Intrinsic Factor – Parietal cells

Code: AD-BAGD

24 tests - For in vitro use only

1. INTENDED USE

Alphadia Biermer Atrophic Gastritis DOT Kit is an Immunodot kit intended for the detection of IgG autoantibodies against the Intrinsic Factor (IF) and Parietal Cell in human serum.

2. INTRODUCTION

Autoimmune gastritis is an organ-specific autoimmune disease characterised by type A chronic atrophic gastritis. The end stage of the disease is pernicious anaemia, which affects predominantly caucasians of north European origin and is considered to be the most common cause of vitamin B12 deficiency in Western countries. Beside typical histological features (e.g. gastric mucosal atrophy), type A chronic atrophic gastritis is characterised by circulating antibodies to parietal cells and Intrinsic Factor (IF).

Parietal Cell Antibodies (PCA): the molecular target of parietal cell antibodies is the H^+/K^+ ATPase, an abundant membrane enzyme which catalyses the countertransport of H^+ and K^+ and is responsible for the acidification of the stomach. Anti-H^+/K^+ ATPase autoantibodies can be detected in 80-90 % of type A chronic atrophic gastritis patients, from early stages to pernicious anaemia, by indirect immunofluorescence and they are also detected in 2-5 % of the adult healthy population.

Intrinsic Factor Antibodies (IFA): anti-intrinsic factor antibodies are strictly restricted to pernicious anaemia and are much more specific markers for this severe outcome. Two types of autoantibodies to IF have been described: Blocking antibodies (Type I) are directed against the binding site of vitamin B12 to IF and prevent the formation of B12-IF complexes, whereas type II antibodies recognise a non-catalytic epitope and may bind indifferently to free intrinsic factor molecules or B12-IF complexes.

ELISA versus RIA methodology: although Radioimmunoassays (RIA) have been used for a long time as reference methods for detecting anti-IF antibodies, they are now advantageously replaced by conventional enzyme immunoassays (EIA : Elisa or Immunodot), known to be more sensitive and specific. Indeed RIA uses radiolabelled vitamin B12 in the test as a competitor for the binding of autoantibodies to IF and therefore is only able to detect type I (blocking) antibodies. Moreover RIA results may be seriously impaired by the interfering presence in the sample oh high levels of unlabelled vitamin B12 (e.g. upon treatment of the patient) or B12-transport proteins (e.g Transcobalamin II) which lead to false positive results. Contrarily EIA methods, which are based on a classical non competitive detection of IF-bound autoantibodies, are able to detect both type I and type II antibodies (increased sensitivity) and are not influenced by the presence in the serum of unlabelled vitamin B12 or B12-absorbing proteins (increased specificity).

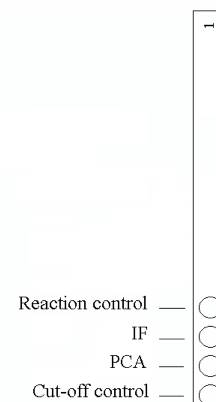
Alphadia Biermer Atrophic Gastritis DOT Kit employs highly purified antigens and combines easy handling and cost effectiveness for the differential diagnosis of Autoimmune Gastritis/Pernicious Anaemia in a single test.

3. PRINCIPLE OF THE TEST

The test is based on the principle of an Enzyme Immuno-assay.

The test strip is composed of a membrane fixed on plastic support. During test procedure, the strips are incubated with diluted patients sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing and AP-conjugated goat antibodies against human IgG are added to the strips. This enzyme conjugate binds to the antigen-antibody complexes. After a second washing step to remove excess conjugate, substrate solution is added.

Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.



4. KIT CONTENTS

Abbreviations :
TBS = Tris Buffer Saline ; BSA = Bovine Serum Albumin ; MIT = MethylIsoThiazolone ;
AP = Alkaline Phosphatase ; NBT = NitroBlue Tetrazolium ; BCIP = Bromo-Chloro-Indolyl-Phosphate.

TO BE DILUTED :

(10 X) Wash buffer : **1 x 50 ml** (colourless)
Contains : TBS, Tween ; Preservative : MIT

READY TO USE :

Dot strips : **24 units**
(4 Dots each: 1 reaction control, 2 antigens, 1 cut-off control)

Diluent buffer : **1 x 40 ml** (yellow)
Contains : TBS, BSA, Tween ; Preservative : MIT

Conjugate : **1 x 40 ml** (red)
Contains : AP-conjugated goat anti-human IgG ; Preservative : MIT

Substrate : **1 x 40 ml** (brown bottle, pale yellow solution)
Contains : NBT/BCIP ; Preservative : 0.05 % NaN_3 (sodium azide)

Incubation trays: **3 units**
(with 8 wells for incubation)

5. MATERIAL REQUIRED BUT NOT PROVIDED

Rocking or shaking platform / Micropipettes / Timer / Graduated cylinder / Distilled or deionised water / Tweezers / Absorbent and/or filter paper

6. STORAGE

The reconstituted Wash Solution is stable for at least one month at 2-8°C. Reagents and strips can be stored at 2-8°C until the expiry date indicated on each vial or tube.
Place unused strips back into the provided tube, seal it and store at 2-8°C. Chromogen/Substrate (NBT/BCIP) shall be stored at 2-8°C.

7. PRECAUTIONS

All reagents are for in vitro diagnostic use only. The kit contains potentially hazardous components thus avoid contact with skin, eyes or mucosae. Patient samples shall be handled as potentially infectious. Do not substitute reagents or mix strips with different batch numbers this may lead to variations in the results. Avoid touching strips with fingers. Use tweezers or wear laboratory gloves. Allow reagents and strips to equilibrate at room temperature before use. Strictly observe incubation times. Handle Chromogen Substrate (NBT/BCIP) with care in order to avoid any contamination with Alkaline Phosphatase.

8. SAMPLE COLLECTION, HANDLING AND STORAGE

Samples should be preferentially fresh-collected ones. Sera with debris should be low speed centrifuged. Blood samples should be collected in dry tubes or in tubes containing EDTA or heparin. After separation serum samples shall be used immediately or aliquoted and stored at 2-8°C for some days or alternatively frozen at -20°C for longer periods. Avoid repeated freezing thawing cycles.

9. ASSAY PROCEDURE

BASIC HANDLING AND TIPS :
The dots are precoloured blue on the strips, ensuring that all antigens have been dotted correctly onto the membrane. This blue coloration disappears during the first step of the procedure. During incubation with the sample a faint pink background coloration appears on the membrane and disappears upon drying at the end of the procedure.
During the procedure, agitation of the incubation tray is necessary to ensure efficient circulation of fluids over the membrane. Any kind of shaking or rocking platform may be used if the amplitude of the movement does not cause spilling out of solutions and cross-contamination between the wells. Agitation speeds ranging from 10 to 60 rpm are suitable, with 20–30 rpm being preferred.
After each filling of the wells with solution, agitate manually the incubation tray to ensure that the strips are completely immersed and to remove air bubbles which may be trapped under the strip. Alternatively, floating strips may be forced into the solution by pushing down (with tweezers or pipette tip)on the upper part of the strip (plastic label zone).
Avoid touching the membrane zone of the strip with fingers, tweezers or pipette tips. Always use the plastic label zone for handling or manipulation. The whole procedure has to be run at room temperature

9.1 Reagents preparation

1. Allow all components to equilibrate at room temperature before use.
2. Dilute the concentrated Wash Buffer 10x with distilled water.
   Prepare 15 ml diluted Wash buffer per strip tested
   Example: 1,5 ml concentrated wash buffer + 13,5 ml distilled water for one strip.

9.2 TECHNIQUE

1. Place one strip per patient into the wells, blue dots facing up.

2. Add 2 ml Wash Buffer to each well. Incubate (shake) for 10 min.
   Upon correct incubation the blue coloration of the dots completely disappears. If not prolong the procedure until the colour of the dots fades completely.

3. Discard solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.

4. Add 1,5 ml Sample Diluent to the wells.

5. Add 10µl patient sample to the wells. Incubate (shake) for 30 min.
   Avoid touching the membrane with the pipette tip. Preferentially dispense the sample in the solution over the upper part of the strip (plastic label zone).
   Note : Steps 4 and 5 can be combined by pre-diluting the sample in a glass or plastic tube (1,5 ml diluent + 10µl patient sample -> Mix -> Add to the well)

6. Wash 3 x 3 minutes with 1,5 ml Wash Buffer (shake).
   Remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.

7. Add 1,5 ml Conjugate to each well. Incubate (shake) for 30 min.

8. Wash 3 x 3 min. with 1,5 ml Wash Buffer (see 6.)

9. Add 1,5 ml Substrate to the wells. Incubate (shake) for 10 min.

10. Wash 1 x 3 min. with 1,5 ml Wash solution to stop the reaction.

11. Collect the strips from the wells and allow them to dry on absorbent paper.

10. RESULTS INTERPRETATION

10.1 Interpretation

1. Peel off the cover of the adhesive on the back side of each strip and attach strips dots face up onto the marked fields of the interpretation sheet provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.

2. Check the first upper Dot (reaction control): it must be positive for all patients.
   Only a clearly coloured positive Reaction Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded.

3. Compare the specific antigen Dots to the cut-off Dot which always is the last in order.
   The colour intensity of the Antigen Dots is directly proportional to the titer of the specific antibody in the patients sample.
   Under optimum conditions and if the sample is free of interfering substances the cut-off dot may be even close to uncoloured. In contrast a highly coloured cut-off Dot indicates a high rate of unspecific binding in the sample.

POSITIVE RESULT: A sample is positive for a specific antibody if the colour intensity of the corresponding Antigen Dot is higher than the intensity of the cut-off Dot.

NEGATIVE RESULT: A sample is negative for a specific antibody if the colour intensity of corresponding Antigen Dot is lower or equal than the intensity of the cut-off Dot.

11. TEST LIMITATIONS

Alphadia Biermer Atrophic Gastritis DOT Kit is intended for the detection of autoantibodies to Intrinsic Factor and Parietal Cell. It serves as an aid for the differential diagnosis of type A chronic atrophic gastritis and pernicious anaemia. Results have to be interpreted in the light of the clinical examination and case history.

12. BIBLIOGRAPHY

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ALPHADIA
AVENUE VESALE 26
1300 WAVRE – BELGIUM
Tel : +32(0)10 24 26 49
Fax : +32(0)10 24 55 99
e-mail : alphadia@euronet.be