Anti-Parietal Cell Antibody Test System

RESULTS
Parietal Cell antibody (PCA) positive results are observed as bright granular cytoplasmic fluorescence of parietal cells of the gastric mucosa. Fluorescence of other cellular antigens such as nucleoli, smooth muscle or connective tissue should not be reported as positive PCA.

TEST LIMITATIONS
1. No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration.

LITERATURE REFERENCES

INTENDED USE
The Bio-Diagnostics Anti-Parietal Cell Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of antibodies to parietal cells in human serum.

SUMMARY AND EXPLANATION
Gastric autoimmune disease has been classified into Type A and Type B gastritis. Patients with antibodies to parietal cells (PCA) or intrinsic factor (or both) have atrophy of the fugal mucosa (Type A). A positive PCA is the presence of a megaloblastic anaemia makes pernicious anaemia a probable diagnosis. In type B gastritis, PCA is lacking and there is no association with pernicious anaemia or other autoimmune endocrine disorders (1). Conditions other than pernicious anaemia may give positive PCA results and in the normal population, PCA varies from 2% in the under 20 age group to 16% in the over 60 age group (2). The gastric mucosa of rat or mouse stomach is used for PCA detection.

PRINCIPLE OF THE TEST
The primary reaction involves circulating antibodies in the patient’s serum, which attach to their homologous smooth muscle antigens. PCA are organ specific and bind to intercytoplasmic components of the parietal cell. Mitochondrial antibody (MA) will also react with parietal cells, resembling PCA fluorescence. To differentiate, a true PCA will not show renal tubular fluorescence on rat kidney tissue while a MA will react with both kidney tubules and parietal cells (3). This occurs during the incubation period while the serum covers the antigen surface.

A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence. With a positive reaction, the nuclear pattern appears apple-green when viewed under a fluorescent microscope, whilst a negative reaction appears black or greenish-black.
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WARNINGS AND PRECAUTIONS
1. All human components have been tested by radioimmunoassay for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the absence of (HBsAg) or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The controls included in the kit contain 0.1% sodium azide as a preservative. Although this is at a low concentration, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
5. For in vitro diagnostic use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artefacts.

KIT CONTENTS

| SLIDE | Rat stomach substrate antigen slides (S4103, S8103, S5103 or S0103) |
| CONJ IgG | FITC Conjugate with Evans Blue Counterstain: J501/J501. This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class. |
| CONTROL + | Parietal cell antibody Positive Control no: C006N/C006N-0.5 |
| CONTROL - | Universal Negative Control no: C000 |
| BA/DFA PBS | Buffer Pack no: R002 |
| MM | Mounting Medium no: R005 |

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes and rack or microtitre system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Distilled Water
Fluorescence Microscope
Volumetric Flask (500 ml)
Paper Towels – lint free

REAGENT PREPARATION

KEY FOR OTHER SYMBOLS
Used in this instruction leaflet and on accompanying product packaging:

- Manufacturer
- Contains sufficient for <n> tests
- RFU Ready for use
- Temperature limitation
- IVD In vitro diagnostic medical device

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STORAGE AND STABILITY
The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.

SPECIMEN COLLECTION
Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipoaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thioumerosal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS
Screening: Dilute test sera 1/20 (1 part patient sample to 19 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

Titration: set up doubling dilutions of serum starting at 1/20, (i.e. 1/20, 1/40, 1/80, 1/160, 1/320, etc.).

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30µl) and controls over the antigen wells.
3. Place slide with patient’s serum and controls in a moist chamber for 30 minutes at room temperature (approximately 18-24°C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Place a blotted on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up the wells to blotter holes. Place slide on top of the blotter. Wipe the back of the slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. Do not allow tissue to dry.
7. Deliver 1 drop (20-30µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.

Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

QUALITY CONTROL
1. Positive and negative serum controls must be included in each day’s testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from non-specific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.