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

1. Vandelli *et al*: Autoantibodies to gastrin producing cells in antral (type B) chronic gastritis. N Engl J Med 300(25): 1406-10, 1979.
2. Strickland R & Mackay I: A reappraisal of the nature and significance of chronic atrophic gastritis. Am J Diag Dis 18:426-40, 1973.
3. Sherlock S and Schever PJ: The presentation and diagnosis of 100 patients with PBC. N Engl J Med 289:647, 1973.

INSTRUCTIONS FOR USE

Parietal Cell Antibody Test System

K4808 - 48 Tests **K9608** - 96 Tests
K5008 - 50 Tests **K0008** - 100 Tests

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 fungal 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.

Bio-Diagnostics Ltd, Upton Industrial Estate, Rectory Road, Upton-upon-Severn, Worcestershire, WR8 0LX, United Kingdom. ☎ +44 (0) 1684 592262 email: enquiries@bio-diagnostics.co.uk

WARNINGS AND PRECAUTIONS

1. All human components in the controls have been tested and found to be negative or non-reactive for STS, HBsAg, HIV 1/2 antibody, HIV 1 Ag and HCV antibody. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The reagents included in the kit contain either 0.1% sodium azide, 0.01% thiomersal or 0.05% ProClin as preservatives. 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/J501-5.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: C000N/C000N-0.5
IFA/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	Volumetric Flask (500 ml)
Distilled Water	Fluorescence Microscope	Paper Towels – lint free

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

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		

Anti-Parietal Cell Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. 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.
Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

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. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening: dilute test serums 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 18-24ºC).
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. Carefully dry the back and edges of the slide using a paper towel. **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.