Trinity Biotech

PARIETAL CELL ANTIBODY TEST SYSTEM

REF 10-4096 **96 Tests**

Store kit at +2 to +8°C

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Para outras línguas
Για τις άλλες λώσσες
För andra språk
For andre språk

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INTENDED USE

These reagents are intended for use in the detection and quantitation of IgG antibody in human sera to parietal cell antigens of stomach tissue by the indirect fluorescent antibody (IFA) procedure. **For *In Vitro* Diagnostic Use.** High Complexity Test

SUMMARY AND PRINCIPLES

Gastric autoimmune diseases have been classified into Type A and Type B gastritis based on the morphological changes of the fundus and antral portion of the stomach. (1) Patients with antibodies to parietal cells (PCA) or intrinsic factor (or both) have atrophy of the fundal mucosa (Type A) and a very high rate of pernicious anemia often associated with other autoimmune endocrine disorders. (2) A positive PCA in the presence of a megaloblastic anemia makes pernicious anemia a probable diagnosis. (3) In Type B gastritis, PCA is lacking and there is no association with pernicious anemia or other autoimmune endocrine disorders.(4)

The indirect immunofluorescent method is the test of choice for detecting PCA as it is more sensitive than the CF method. The gastric mucosa of the rat stomach is the most commonly recommended substrate employed in the detection of PCA.

The incidence of PCA in patients with pernicious anemia is 93%. Conditions other than pernicious anemia may give positive PCA results: atrophic gastritis, diabetes mellitus, Hashimoto's disease, gastric ulcer, thyrotoxicosis, myasthenia gravis, iron deficiency anemia, idiopathic Addison's disease, primary myxedema, Sjogrens syndrome and rheumatiod arthritis. In normal population, PCA varies from 2% in under 20 age group to 16% in the over 60 age group.

PCA should be included in a differential work-up of patients megaloblastic anemia since 93% of patients with pernicious anemia will be detected.

PRINCIPLES

The PCA reaction involves circulating antibodies to intercytoplasmic components of the parietal cell. PCA is organ specific, but not species specific. However, antimitchondrial antibody (MA) is not organ specific and will react with parietal cell and resemble PCA fluorescence. Therefore, in order to differentiate a true PCA from a MA the specimen showing PCA fluorescence should be tested on rat kidney section. A true PCA will not show renal tubular fluorescence while a MA will react with both kidney tubules and parietal cells.

Recent studies have demonstrated a potential pitfall in the detection of PCA. Smooth muscle antibodies (SMA) from patients with chronic acute hepatitis (CAH) bind to gastric parietal cells in an immunofluorescent pattern indistinguishable from PCA. Therefore, in order to differentiate a true PCA from a SMA, the specimen showing PCA fluorescence should be checked for a positive staining in muscularis mucosa. A true PCA will not show the stomach muscularis muscosal fluorescence, but a SMA may react with both muscularis mucosare and parietal cells. (5)

PCA is primarily IgG but may occasionally be found in IgM immunoglobulin fractions.

The primary reaction involves circulating PCA antibodies present in the patient's serum which attach to their homologous parietal cell antigens. This occurs during the incubation period while the serum covers the antigen surface. A secondary reaction utilizing a fluorescein labeled anti-human conjugate then follows a rinsing period which removes all unbound human antibody. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescence microscope. Bright granular cytoplasmic fluorescence limited to the parietal cells of the rat stomach gastric mucosa indicates a positive result. Fluorescence of other cellular antigens such as nuclei, smooth muscle, connective tissue or chief cells should not be reported as positive PCA.

MATERIALS PROVIDED

Prod#	Description	Quantity
10-3008	Rat Stomach, 8 well slide	12 ea
10-4202	Parietal Cell Positive Control	0.5 ml
10-1201	Autoimmune Negative Control	0.5 ml
10-1501	FITC IgG Conj. Rodent Ads. w/ Evans' Blue	4.0 ml
90-1610	FITC Mounting Medium (pH 7.5)	3.0ml
90-1607	Phosphate Buffered Saline (pH 7.5)	2x10 g
90-1700	Coverslips 70x22 mm	12 ea
90-1708	Blotters, 8 well	12 ea

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Test tubes, test tube rack, pipettes.
2. Volumetric flask (1000 ml).
3. Staining dish.
4. Fluorescence microscope.
5. Microscope Slide Roller.

STORAGE AND STABILITY

1. Antigen slides Prod# 10-3008 should be stored at +2 to +8°C. Slides are stable until their expiration date on the product label.
2. Positive control Prod# 10-4202 should be stored at + 2 to +8°C. Refer to expiration date on label.
3. Negative control Prod# 10-1201 should be stored at + 2 to +8°C. Refer to expiration date on label.
4. FITC labeled anti-human conjugate Prod# 10-1501 should be stored at +2 to +8°C. Refer to expiration date on label.
5. Mounting Medium Prod# 90-1610 should be stored at +2 to +8°C. Refer to the expiration date on label.
6. Phosphate buffered saline pH 7.5 Prod# 90-1607 are stable at room temperature. Reconstitute each vial of PBS buffer salts with 1.0L of distilled water. The PBS contains no preservative and should be stored at +2 to +8°C. Discard if turbidity develops.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2°C to 8°C if it is to be analyzed within 4-7 days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium azide is strongly recommended. The CLSI provides recommendations for storing blood specimens (Approved Standard Procedure for the Handling and Processing of Blood Specimens, H18-A2 2005). (9)

TEST PROCEDURE

Dilute test serums 1:20 in PBS if testing is being performed for screening purposes. For titrations set up doubling dilutions of serum starting at 1:20, (i.e. 1:20, 1:40, 1:80, 1:160, 1:320,etc). The slide, controls and conjugate are ready to use.

1. Tear 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 (15 to 20ul) over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature. (approximately 20°C)
4. Remove slide from moisture chamber. Using a wash bottle, gently rinse remaining sera from slides being careful not to aim the stream directly on the well.
5. Wash in PBS for two separate five minute changes.
6. Remove the slides from PBS and place slide antigen side facing up on a dry paper towel. Carefully place the blotter over the slide so that the blotter is indexed to the surface of the microscope slide. Hold one edge of the blotter with one hand to keep the blotter in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture between the antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
7. Using dispenser provided*, deliver 1 drop (25ul) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70mm cover glass. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a humid chamber placed in a dark refrigerator.

* The conjugate dispenser is provided with a calibrated tip and allows quantitative delivery of reagents from the storage bottle. To use, wipe the tip with a paper towel, invert the bottle and squeeze gently to release one drop. If the tip contains an air bubble, tap the bottle gently to remove air bubble which will ensure precise drop delivery.

RESULTS

Pernicious anemia is a megaloblastic anemia. A positive PCA test from a patient with a megaloblastic anemia helps establish a Presumptive diagnosis of pernicious anemia or pernicious anemia associated with a second disease. Additional confirming tests for pernicious anemia are: antibodies to intrinsic factor, vitamin B12 absorption or serum vitamin B12 activity. A key factor in differentiating between pernicious anemia and simple atrophic gastritis is the lack of antibody to intrinsic factor in atrophic gastritis.

On the basis of PCA alone one may assume some form of atrophic gastritis which may or may not be related to pernicious anemia. PCA are generally associated with some degree of hypochlohydria.

In addition to its diagnostic potential PCA testing is helpful in screening genetically determined high risk groups (ie. relatives of thyroid patients and pernicious anemia patients) for asymptomatic chronic atrophic gastritis and for early recognition of atrophic gastritis and pernicious anemia.

A positive result is observed as bright granular cytoplasmic fluorescence of parietal cells of the rat gastric mucosa. Fluorescence of other cellular antigens such as nuclei, smooth muscle, or connective tissue should not be reported as positive PCA.

TITER INTERPRETATION

The titer is the highest dilution of the patient's serum showing weak 1+ fluorescence of the parietal cell.

"The clinical significance of the PCA titer has no relation to the severity or duration of the disease state. Thus, one cannot predict or assume on the basis of PCA titer alone the degree of impaired secretion of intrinsic factor or the extent of histopathologic changes." (Immunofluorescence detection of autoimmune disease. Immunology Series No.7, U.S.D.H.E.W.CDC.1976.p66).

LIMITATIONS OF PROCEDURE

1. No diagnosis should be based upon a single serologic test result, since various host factors must be taken into consideration.
2. Additional confirming tests for pernicious anemia are: antibodies to intrinsic factor, vitamin B12 absorption or serum vitamin B12 activity.
3. PCA should be used as a diagnostic aid in establishing pernicious anemia as the cause of megaloblastic anemia.
4. PCA can be found in 16% of apparently normal individuals over the 60 year age group.
5. Conditions other than pernicious anemia may give positive PCA results.
6. The presence of intrinsic factor autoantibodies is considered to be diagnostic for pernicious anemia and for rare cases of endocrine disorders associated with gastric atrophy. (6)
7. Patients with dermatitis herpetiformis can have PCA without any evidence of malabsorption of B12.

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 The negative serum control should result in little(+) or no fluorescence of the nuclei. If this control shows bright fluorescence either the control or the antigen may be at fault.
3. The positive serum control should result in 3+ to 4+ fluorescence of the type specified on the label. If this control shows little or no fluorescence either the control, antigen, conjugate or technique may be a fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific 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 the antigen may be at fault.

PRECAUTIONS

1. Always wear suitable protective clothing, gloves and eye/face protection when working with this product.
2. Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV as well as for HBsAg and found to be negative (were not repeatedly reactive). WARNING - POTENTIAL BIOHAZARDOUS MATERIAL Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1999. (8)
3. Some components in this kit contain 0.02% Thimerosal. Thimerosal is toxic by inhalation, in contact with skin, and if swallowed, and is a reproductive hazard.
4. All reagents must be brought to 20 to 25°C before performing the test procedure.
5. Some components contain sodium azide at a concentration of less than 0.1%. Sodium azide is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
6. The phosphate buffered saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.
7. Some components in this kit contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitisation by skin contact.
8. The conjugate in this kit contains 0.015% Evan's Blue. Evan's Blue is a possible carcinogen and may cause reproductive harm.
9. Do not use components beyond their expiration date.
10. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
11. For in vitro diagnostic use.
12. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
13. Once the procedure has been started do not allow antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

The safety data sheet is available upon request.

WARNING
Some components of this kit contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
H317: May cause an allergic skin reaction.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING
Some components of this kit contain < 0.1% sodium azide.
H302: Harmful if swallowed.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P501: Dispose of contents/container in accordance to local, regional, national and international regulations.

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8. Centers for Disease Control/National Institutes of Health (CDC-NIH) Manual. 1999. In: *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition, U.S. Dept. of Health and Human Services, Public Health Service.
9. Clinical Laboratory Standards Institute (CLSI). 2005. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline – Second Edition. CLSI Publication H18-A2.

Consult Instructions for Use

REF	Product Number
LOT	Lot Number
IVD	*In Vitro* Diagnostic Medical Device
EC REP	Authorized Representative in the European Community
	Use By
	Caution, consult accompanying documents
	Temperature limitation
	Manufacturer
 or 	WARNING
CONTROL -	Negative Control
CONTROL PC +	Parietal Cell Positive Control
CONJ	Conjugate
PBS	Phosphate Buffered Saline
MTMED	Mounting Medium
CVRSLP	Coverslips
BLTRS 8W	Blotters, 8 Well
SLD 8W	Slide, 8 Well

EC REP-Trinity Biotech plc.
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