Introduction:

Gastric autoimmune disease have been classified into Type A and Type B gastritides, 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 and is more sensitive than the CF method. The gastric mucosa of rat or mouse stomach is utilized for PCA detection in this test system.

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, Sjögren's syndrome and rheumatoid arthritis. In the normal population, PCA varies from 2% in the under 20 age group to 16% in the over 60 age group.

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

Principles:
The Parietal Cell Antibody reaction involves circulating antibodies to intercytoplasmic components of the parietal cell. PCA is organ specific, but not species specific. However, anti-mitochondrial antibody (MA) is not organ specific and will react with parietal cells and resemble PCA fluorescence. therefore, in order to differentiate a true PCA from an MA, the specimen showing PCA fluorescence should be tested on rat or mouse kidney section. A true PCA will not show renal tubular fluor-escence while an 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 the muscularis mucosa. A true PCA will not show the stomach muscularis mucosal fluorescence, but a SMA may react with both muscularis mucosa and parietal cells.[5]

In order to facilitate this type of differentiation, slides are available containing three sections per well, i.e.; monkey thyroid/rat stomach/rat kidney - catalog # 2354, which allows

for immediate differentiation of thyroid reactions, as well as PCA from non-organ specific MA reactions in one well. PCA is primarily IgG, but may occasionally be found in the 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 then follows a rinsing period, which removes all unbound human antibody. The reagent used in the secondary reaction is a fluorescein labeled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescence micro-scope. Bright granular cytoplasmic fluorescence limited to the parietal cells of the rat or mouse stomach gastric mucosa indicates a positive result. Fluorescence of the other cellular antigens such as nuclei, smooth muscle, connective tissue or chief cells should not be reported as positive PCA.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1501L (3.0 ml)/15015L (5.0 ml) with Evans Blue Counterstain is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent contains antibodies, which will react with the human IgG, IgM and IgA Immunoglobulin classes.
2. The antigen slides of rat stomach sections must be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.
3. PCA positive control No. 4202L (1.0 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage as indicated on label. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.
6. Mounting Medium No. 1610 is stable when stored at 2-8°C. Check label for specific expiration date.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601. Rehydrate buffer with 1 liter of sterile distilled water.

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 2-8° C if it is to be analyzed within a few 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, addition of a preservative such as 0.095% sodium azide is strongly recommended.

Test Instruction:

Screening: dilute test serums 1:20 in PBS. **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 30 µl) 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 19 - 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 on to the well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotter 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 wells to blotter holes. Place slide on top of blotter. Do not allow tissue to dry. Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
7. Deliver 1 drop (25-30 µl) 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. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at 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 antigen may be at fault.

Results:

Pernicious anemia is 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 atrophia 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 hypochlorhydria.

In addition to its diagnostic potential, PCA testing is helpful in screening genetically determined high risk groups (i.e.; 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 or mouse 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+ florescence 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. Clinical manifestations, histologic finds on liver biopsies, elevation of IgM and increased alkaline phosphatase values should all be considered in the final diagnosis of PBC.
3. Liver and kidney microsomal antibody stains proximal tubules preferentially whereas MA reacts with distal tubules more strongly than with proximal tubules.
4. A normal serum IgM is strong evidence against the diagnosis of PBC as increased concentration of this immunoglobulin is the dominant abnormality in this disease.
5. Anti-smooth muscle antibody can be detected in 30-50% and antinuclear antibody in 25-46% of patients with PBC.

Precautions:

1. All human components have been tested by radio-immunoassay for (HB_sA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_sA_g or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to insure 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 artifacts.

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