

DETECTION OF ANTI-INTRINSIC FACTOR ANTIBODIES BY A NEW NON-RADIOACTIVE ELISA-BASED ASSAY

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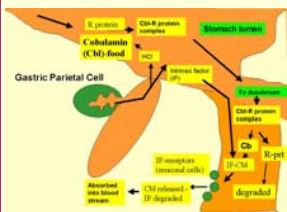
Abstract

Objective: Evaluate the performance of a new non-radioactive ELISA assay for the detection of anti-intrinsic factor antibodies.
Methods: A combined cohort of 676 specimens obtained from individuals with definite or presumed pernicious anemia (95), suspected pernicious anemia (82), various non-pernicious anemia diseases (23), and 476 specimens from healthy controls were tested for anti-intrinsic factor antibodies by the Quanta Lite™ Intrinsic Factor Antibody ELISA (INOVA Diagnostics). This assay uses recombinant human intrinsic factor as the substrate and in contrast to RIA methods, detects both type 1 and 2 intrinsic factor antibodies. Laboratory indicators of pernicious anemia included hematological examination, mean corpuscular volume (MCV), vitamin B12 levels, and gastric parietal cell antibody (GPA). Determinations were not available for every specimen. Results were correlated with available clinical data and results obtained with other tests. Two groups of specimens submitted to commercial reference laboratories for GPA testing only were also tested for the presence of intrinsic factor antibodies.

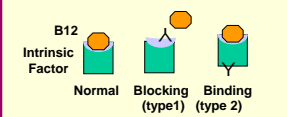
Results: Anti-intrinsic factor antibodies were detected in 96.8% (92 of 95) of specimens which had laboratory evidence of pernicious anemia. Two additional specimens were interpreted as equivocal. A direct comparison of the Quanta Lite™ results with those obtained by an RIA-based intrinsic factor antibody assay (Diagnostic Products, Inc.) on a panel of gastric parietal cell antibody positive specimens showed 95.6% (66/69) agreement between the two assays. All specimens found positive by both the RIA and ELISA kits had evidence of pernicious anemia. In contrast, while all specimens sent for intrinsic factor antibody testing at a US reference laboratory which were found positive by the INOVA ELISA assay were also positive by the RIA (DPC) assay, almost 70% of the specimens interpreted as positive by the RIA test were negative by the ELISA test. False positive RIA results could result if patients had received therapeutic doses of vitamin B12. Over 90% of the specimens positive for Intrinsic Factor Antibody were also positive for GPA. On a cohort of patients under suspicion of pernicious anemia, about 50% of the GPA positive specimens were also positive for intrinsic factor antibody. Examination of specimens sent to 2 reference labs specifically for GPA antibody testing showed that about 21% of specimens with positive GPA results were also positive for intrinsic factor antibodies.

Conclusions: The Quanta Lite™ Intrinsic Factor Antibody ELISA offers a non-radioactive alternative to RIA-based assays, detects both type 1 and 2 intrinsic factor antibodies, and is not affected by vitamin B12 levels. In addition to hematological consequences, patients with pernicious anemia are at risk of neurological problems and increased risk of some cancers. The availability of this assay should encourage more extensive testing for intrinsic factor antibodies and may result in the identification, treatment, and management of more patients with pernicious anemia.

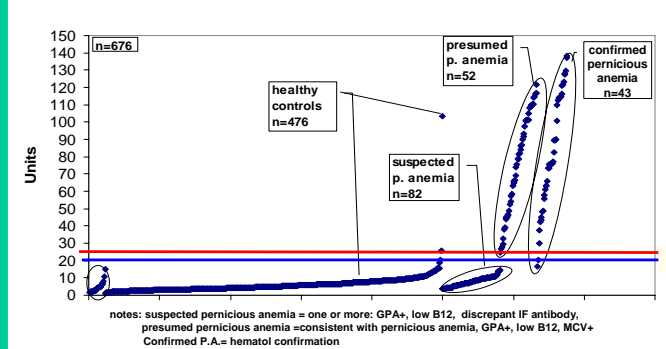
Vitamin B12 Absorption



Intrinsic Factor Antibodies



Quanta Lite Intrinsic Factor Antibody ELISA



notes: suspected pernicious anemia = one or more: GPA+, low B12, discrepant IF antibody, presumed pernicious anemia = consistent with pernicious anemia, GPA+, low B12, MCV+ Confirmed P.A.= hematol confirmation

Sensitivity & Specificity

Clinical Sensitivity : 96.8% (92/95)
Positive % agreement INOVA IF ELISA and DPC RIA:
 site 1= 95.6% (66/69) [all GPA+, clinical diagnosis of P.A.]
 site2 = 30.7% (23/75)
Specificity (healthy & disease controls): 99.6% (497/499)

Testing for GPA only misses Intrinsic Factor+ individuals

Clin. Lab 1= 21.4% (3/14) of GPA+ spec. were IF pos
Clin. Lab 2= 26.1% (6/23) of GPA+ spec. were IF pos

Background

Pernicious anemia is a chronic disease and the end-stage of type A (autoimmune) chronic atrophic gastritis and is the most common cause of vitamin B12 deficiency. During the progression of type A chronic atrophic gastritis (up to 20-30 years), gastric parietal cells, which produce intrinsic factor and HCl, and zymogenic cells, which produce pepsinogen, are destroyed and production of intrinsic factor (IF) and HCl is eliminated. Intrinsic factor is essential for the absorption of vitamin B12 from the intestine and its absence leads to vitamin B12 deficiency and megaloblastic anemia. Diagnosis of pernicious anemia is important for treatment of the anemia itself and prevention of irreversible neurological damage. Patients with pernicious anemia have a 3 times increased risk of gastric carcinoma, a 13 times increased risk of gastric carcinoma, and an increased risk of esophageal squamous cell cancer. Circulating antibodies to Intrinsic Factor are highly specific and can be detected in >50% of patients with pernicious anemia. These antibodies are of 2 types: Type 1 blocking antibodies which prevent the binding of vitamin B12 to the IF molecule and Type 2 antibodies which may interfere with the binding of the IF- vitamin B12 complex to the ileal receptor. Together with other clinical and laboratory data, a positive intrinsic factor antibody result can help distinguish pernicious anemia from other megaloblastic anemias as well as distinguish type A atrophic gastritis from other forms of nonspecific histological gastritis.

Since 40% of pernicious anemia patients may be negative for IF antibodies, a negative result does not rule out the disease. Gastric parietal cell antibodies are present about 90% of individuals with pernicious anemia. Ideally both gastric parietal cell and intrinsic factor antibodies should be evaluated for optimal evaluation of an individual's status.

Intrinsic factor antibodies are measured by radioimmunoassay (RIA) in many US laboratories. In addition to issues associated with the use of radioactivity, RIA can give false positive results if individuals receive therapeutic doses of vitamin B12 and false negative results if only type 2 antibodies are present. ELISA assays, such as the one described in the present study, do not suffer from this problem.

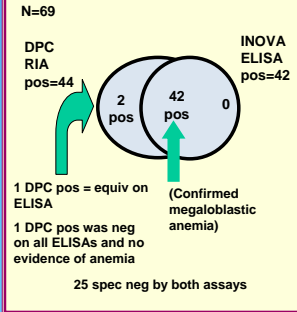
Methodology

QUANTA Lite™ Intrinsic Factor ELISA
 • Recombinant human intrinsic factor antigen.
 • Patient specimens are run at a 1:101 dilution
 • Pre-diluted controls, single-point antigen specific calibration, 30 minute room temperature incubations, ready-to-use conjugate, and single vial TMB substrate solution. Results expressed in arbitrary units.
Cutoff Establishment for the QUANTA Lite™ Intrinsic Factor ELISA
 A panel of 476 specimens collected from healthy individuals and 23 specimens from patients with a variety of non-pernicious anemia diseases was tested with the Intrinsic Factor ELISA to establish the cutoff for the assay. The specificity of the assay was 99.4% (473/476) for healthy controls and disease control sera. Excluding the one strong positive result outlier, the mean and median values for the healthy control group were 5.7 and 5.5 units respectively.

Grouping of Sera Examined

Suspected Pernicious Anemia = one or more tests positive: gastric parietal cell antibody, low vitamin B12, but intrinsic factor antibody results were discrepant
Presumed Pernicious Anemia = laboratory measures consistent with pernicious anemia including gastric parietal cell antibody positive, low vitamin B12, mean corpuscular volume (MCV) increased (typical of pernicious anemia)
Confirmed Pernicious Anemia: laboratory measures consistent with pernicious anemia including gastric parietal cell antibody positive, low vitamin B12, mean corpuscular volume (MCV) increased (typical of pernicious anemia), hematological confirmation of megaloblastic anemia

Luxembourg Study Cohort



Conclusions

- 21-26% of the GPA+ specimens sent to 2 clinical laboratories were also found to be intrinsic factor positive. The high specificity of the Intrinsic factor antibody test suggests that routine testing for GPA and Intrinsic factor antibodies may be a reasonable course.
- Sensitivity was 96.8% on a panel of sera from patients with pernicious anemia.
- All INOVA Intrinsic factor ELISA positive specimens were also RIA positive, but not vice versa. Most discrepant sera were GPA negative.
- The Quanta Lite Intrinsic Factor ELISA has been submitted to the FDA for 510(k) review and clearance.

References

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