each specimen in the group is combined in a separate tube, thereby giving a mixture of 10 samples each at a 10-fold dilution. In addition, an aliquot of this mixture is further diluted 10-fold for a final 100-fold dilution. Each specimen and the two dilution pools are then analyzed. If no sample showing the hook effect is in the group, the dilution-corrected results from the 10-fold and 100-fold dilution pools are essentially the same. However, if a hook effect sample is present, the 100-fold dilution pool returns a much higher result than does the 10-fold dilution pool. For example, when a patient specimen with a known PSA concentration of >35 000 μg/L was analyzed, the 10-fold dilution pool returned a value of 558 μg/L, but the 100-fold dilution pool returned a value of 26 780 μg/L. Discrepancies such as this indicate to the technologist the need to reanalyze each of the 10 specimens in the group at greater dilutions to identify the specimen showing the hook effect. This strategy requires the routine analysis of two additional pool specimens for every 10 patients' specimens for an overall analytical cost increase of only 20%, but also ensures that specimens with grossly increased concentrations of tumor markers will not be misanalyzed. To further reduce costs, we have also used this approach for ≤30 patients' specimens in a single pool and were able to identify a hook effect specimen with a true PSA concentration of 20 000 μg/L at an additional cost of only ~7% for the original screening. This strategy represents a cost-effective way to ensure the identification of specimens with extremely high concentrations of PSA or other tumor markers; however, because of differences in the concentration at which the hook effect analyte exhibits the hook effect and differences in analytical methods, a laboratory needs to establish its own optimum pool size for each tumor marker assay.

References

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Increased Concentrations of Fecal Anti-Gliadin IgA Antibodies in Untreated Celiac Disease

To the Editor:
The diagnosis of celiac disease (CD) is based on the detection of subtotal or total villous atrophy in jejunal biopsies (1). The detection of serum anti-gliadin IgA antibodies (AGA IgA) was recently proposed for the selection of candidates for biopsy (2). Serum AGA IgA contains some polymers that may be of intestinal origin (3, 4). These results suggested the involvement of both systemic and intestinal immunity in the production of AGA IgA in CD. Intestinal humoral immunity is mainly mediated by polymeric IgA, which resists proteolysis by association with the secretory component and is found in the feces whole or as fragments, often bound to α1-antitrypsin or pigments (5-7). We have thus developed a noninvasive method based on an assay of fecal AGA IgA to investigate intestinal humoral immunity. Our aim was to demonstrate the presence of AGA IgA in feces of patients with CD and to compare this variable with serum AGA IgA. By adapting the method proposed by Juto et al. (8) to fecal samples, we sought fecal AGA IgA in patients with untreated CD.

Three-day stools mixed with 1 g/L sodium azide and 1 mmol/L diisopropylfluorophosphate were stored at 4°C. Fecal protein was extracted from 5 g of the homogenate with 10 mL of 0.15 mol/L NaCl for 1 h at 4°C. The supernatant obtained by centrifugation at 10 000 × g for 10 min was stored at −20°C.

AGA IgA was determined by using a sandwich-type ELISA method (Gluten IgA EIA*, Pharmacia, Uppeala, Sweden). The optimal dilution (60-fold dilution to 0.15 mol/L NaCl) was determined by adding AGA IgA to normal fecal samples. Fecal extract (100 μL) was incubated for 1 h at 37°C in multiwell microplates coated with crude gliadin. The following steps were performed according to the manufacturer's instructions. Results are expressed in arbitrary units (AU) determined as the 405 nm absorbance ratio between the sample and an AGA IgA-positive reference serum.

The assay was linear between 10 and 120 AU, with a detection limit of 1 AU. Within-run variation, determined with fecal extracts, was 4.5% (n = 10); between-run variation was 10% (n = 7). The study of healthy subjects gave a positivity threshold of 10 AU for fecal extracts and 25 AU for serum samples.

Fecal and serum AGA IgA were determined in a group of patients with untreated CD and in a control group of healthy subjects. The patients consisted of 11 women and 8 men (mean age 44 ± 21 years) with jejunal biopsy-proven villous atrophy (flattened mucosa) (9). Of the 19 patients, 14 improved clinically (and on repeat biopsy) after introduction of a gluten-free diet (GFD); the remaining 5 were lost to follow-up after introduction of a GFD. The control group consisted of six men and six women (mean age 39 ± 15 years) with no major gastrointestinal disturbances and with normal fecal nitrogen and lipid contents and intestinal α1-antitrypsin clearance. The specificity of the assay, determined in the control population, was 100% and the sensitivity 74% (14 of 19). Further studies are now in progress to evaluate the response of patients with other gastrointestinal diseases.

The median values of AGA IgA in serum (44 AU) and feces (24 AU) in the patient group were significantly higher (P <0.01) than those in the control group (2 AU and 4.5 AU, respectively; Figure 1). Among the patients, 11 had increased concentrations of both serum and fecal AGA IgA, whereas 3 had isolated increases of serum concentrations and 3 had isolated increases of fecal concentrations. Two patients, without IgA deficiency, did not have significantly increased AGA IgA concentrations, compared with the control subjects, in either the serum or feces.

Our results confirm previous reports of the presence of IgA in feces (5-7) and show the value of fecal AGA IgA detection in CD. Like Colombel et al. (4), who analyzed both jejunal fluid
and serum in untreated patients with CD, we found a good correlation (P < 0.01) between serum and fecal concentrations of AGA IgA. Because fecal AGA IgA concentrations reflect the secretion of these antibodies by the whole digestive tract, our results suggest a pathophysiological relation between serum and fecal AGA IgA. Moreover, the emergence of fecal AGA IgA in some patients might precede that of serum AGA IgA during the onset of CD, because three subjects with CD had AGA IgA but normal serum AGA IgA. Gliadin, an antigen found in food, could stimulate intestinal immunity and give rise to detectable fecal AGA IgA even when serum concentrations are zero, either because of inadequate assay sensitivity or because of an absence of AGA IgA.

Combined assay of serum and fecal AGA IgA could prove to be a more precise and thorough means of studying systemic and intestinal humoral immune responses in celiac disease.

References

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Strength of the Signal, Analytical Variability, and Predictive Value of Test Results

To the Editor:

The Bayesian approach, popularized by Galen and Gambino (1), is valuable for assessing diagnostic tests and managing laboratory reports. This approach is of particular importance in judging the reliability of indications of the presence (D) or absence (D) of disease provided by a test result. In the most general (and most frequently adopted) formulation of Bayes’ theorem, numerical estimates reduce to a binary classification (yes-no, positive-negative), thus sacrificing to the mod-