
Influence of Delay in Stool Sampling on Fecal Occult Blood Test Sensitivity, Graeme P. Young,1* Marc A. Sinatra,1 and D. James B. St. John 2

Hemoccult is not stable in stools. The globin molecule is steadily degraded and the heme molecule is modified [1–5]. The latter effect is relevant to fecal occult blood tests that are based on the pseudoperoxidase activity of heme. This peroxidase activity depends on the presence of an iron atom within the porphyrin ring [5–7]. Colonic bacteria can remove the iron atom during colonic transit; furthermore, evidence indicates that removal of the iron atom can continue after passage of a stool [7]. Therefore, the manufacturers of most guaiac-based fecal occult blood tests recommend that samples be taken from freshly passed stools and used, immediately, to form thin smears on a test card, as is the case with the Hemoccult® test (SmithKline Diagnostics, San Jose, CA) tests. However, clinical studies have not always followed this recommendation [8], and the same is probably also true in general pathology laboratories.

To examine the effect of delaying smearing vs immediate smearing of fecal samples onto test cards for the Hemoccult II® and Hemoccult II SENSA® guaiac-based tests, we supplemented fresh stool specimens from five healthy individuals with whole-blood lysate to give various hemoglobin concentrations. Samples were taken from the supplemented specimens without delay after preparation, one test card being prepared for each test for each time point at which the tests were to be subsequently developed. The remainder of each supplemented stool was placed in a standard specimen jar, sealed, and left at room temperature (19–22 °C) to provide samples for delayed testing. Test cards were prepared from the contents of the specimen jars and developed at the same time points as were used with the cards that had been prepared immediately after supplementation.

Test cards were developed and read in bright, direct lighting, the card being viewed for a full 60 s after the addition of developer. Any blue color, no matter how transient, was recorded as positive. The intensity of blue color in each window was graded as follows: 0 for no color, 1 for a trace of blue, 2 for an intermediate degree of blue, and 3 for bright blue covering an extensive area [9]. For each test card, a graded score was derived for the pair of windows by averaging. The person developing the test was unaware of the hemoglobin concentration and the nature of storage of the test cards and fecal samples.

Hemoccult II SENSA was slightly more sensitive for blood added to feces in vitro than Hemoccult II. Threshold analytical sensitivities for hemoglobin in each stool sample (µg/g feces) were 200, 200, 200, 300 for Hemoccult II SENSA and 200, 200, 300, 500 for Hemoccult II when the test cards were developed after immediate smearing.

Table 1 shows the test scores for each of three hemoglobin concentrations, for each occult blood test, when smearing was immediate or delayed. In general, the intensity scores for Hemoccult II SENSA were greater than those for Hemoccult II, and both scores increased as the concentration of added hemoglobin increased. Importantly, from day 2 onwards, for each concentration of hemoglobin, higher scores were obtained for cards that had been smeared with feces directly after supplementing than for those in which the smearing was delayed.

Thus, Hemoccult II SENSA has a higher analytical sensitivity than Hemoccult II for blood in feces, confirming the results in a previous study where the same tests were compared by hospital ward staff [9]. This increased analytical sensitivity translates into improved clinical sensitivity for colorectal neoplasia [10]. However, it is critically important for sensitivity that the samples be stable.

The present study demonstrates a loss of sensitivity when the smearing of feces onto the test card is delayed. Hemoccult II and Hemoccult II SENSA give stable results for at least 19 days when cards are smeared immediately; however, if the supple-

<table>
<thead>
<tr>
<th>Test</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>10</th>
<th>15</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb added, 200 µg/g</td>
<td>0.2/0.2</td>
<td>0.4/0.1</td>
<td>0.4/0.0</td>
<td>0.4/0.0</td>
<td>0.3/0.0</td>
<td>0.0/0.0</td>
<td>0.3/0.0</td>
</tr>
<tr>
<td>Hemoccult II</td>
<td>0.7/0.6</td>
<td>0.8/0.3</td>
<td>1.0/0.4</td>
<td>0.6/0.2</td>
<td>0.7/0.2</td>
<td>0.6/0.0</td>
<td>0.2/0.0</td>
</tr>
<tr>
<td>Hb added, 400 µg/g</td>
<td>1.2/1.2</td>
<td>1.2/0.1</td>
<td>1.2/0.2</td>
<td>1.5/0.0</td>
<td>1.4/0.0</td>
<td>1.3/0.0</td>
<td>1.4/0.0</td>
</tr>
<tr>
<td>Hemoccult II</td>
<td>1.7/1.6</td>
<td>1.9/0.6</td>
<td>1.6/0.5</td>
<td>1.6/0.2</td>
<td>1.5/0.2</td>
<td>1.5/0.2</td>
<td>1.2/0.2</td>
</tr>
<tr>
<td>Hb added, 1000 µg/g</td>
<td>2.3/2.5</td>
<td>2.2/0.9</td>
<td>2.0/0.7</td>
<td>2.2/0.4</td>
<td>2.3/0.1</td>
<td>2.5/0.1</td>
<td>2.3/0.2</td>
</tr>
<tr>
<td>Hemoccult II</td>
<td>2.9/2.9</td>
<td>3.0/1.6</td>
<td>3.0/0.9</td>
<td>2.6/0.6</td>
<td>2.8/0.4</td>
<td>2.7/0.4</td>
<td>2.5/0.2</td>
</tr>
</tbody>
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*(From 0 (negative) to 3 (strongly positive); see text.
9, cards smeared immediately after fecal supplementation with hemoglobin; L, cards smeared later after supplementation and tested 24 h after smearing.)
mented fecal sample is stored in moist form, rather than as a dry smear on a test card, and sampling is delayed, the positivity score falls rapidly. Apparently, the degradation of hem on the removal of the iron atom \[5,7\] is more rapid in moist samples than in the thin, relatively dry smears on the sample cards. Thus, erroneous results (false negatives) will tend to occur when smearing of stools onto the sample collection device is delayed.

This finding is particularly relevant to two areas. First, pathology laboratories that receive stools in an unaltered moist form are likely to miss pathological amounts of blood because of bacterial degradation of the heme. Second, studies in which occult blood testing is performed on moist samples sent to the laboratory, with a resulting delay in smearing of the test card, may not give reliable estimates of the clinical performance of these tests, e.g., in screening for colorectal neoplasia.

We conclude that the test cards for fecal occult blood tests based on the pseudoperoxidase activity of heme should be prepared immediately after defecation. Transport of moist samples to the laboratory should be avoided; that procedure results in reduced sensitivity.

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References

Nonisotopic Detection of Point Mutations in CYP21B Gene in Steroid 21-Hydroxylase Deficiency, Begona Esquieza, *José M. Varela, † Carlos Jariego, *Antonio Oliver, * and Ricardo Gracia* (Depts. of †Biochem. and *Pediatr. Endocrinol., Hosp. La Paz, Paseo Castellana 261, 28046 Madrid, Spain; *author for correspondence: fax 34 1 3582733)

Classical steroid 21-hydroxylase (21-OH; EC 1.14.99.10) deficiency is the enzymatic defect underlying 90–95% of congenital adrenal hyperplasias. The affected enzyme, cytochrome P450c21, is encoded by the gene CYP21B, which is found in the human leukocyte antigen (HLA) class III gene region on chromosome 6p21.3, together with a nonfunctional pseudogene [1]. HLA genotyping has been used to detect 21-OH deficiency carriers and perform prenatal diagnosis in families with a previously haployped affected child, but de novo mutations or intra-HLA recombination has sometimes invalidated the approach [2]. Frequent homozygosity and poor expression of some of these markers in amniocytes have made prenatal diagnosis difficult [3]. Molecular identification of these markers after easy PCR techniques has only been developed for the class II genes. Prenatal diagnosis and carrier detection have also been performed through biochemical evaluation of 17-OH progesterone (17OHP), either in amniotic fluid or after corticotropin (adrenocorticotropic hormone; ACTH) stimulation, respectively. Unfortunately, dexamethasone treatment to the mother, to prevent prenatal virilization, suppresses 17OHP in amniotic fluid [4], and the results for post-ACTH 17OHP carriers overlap those of controls [1, 3, 5].

Direct analysis of the gene is now feasible and offers a more accurate identification of patients and carriers in affected families [6–9]. Moreover, the strong genotype–phenotype relationship found in this disease [7–9] reinforces interest in finding the mutations underlying this deficiency. The severity of the disorder is related to the degree of impairment of the enzymatic activity, which depends, in turn, on the severity of the mutation. In addition, we must consider that patients with mild forms of the deficiency may carry severe mutations [7–9] that will not be detected by methods other than direct gene analysis (see below, Fig. 1A).

Point mutations in the CYP21B gene are the molecular defect in 70% of the alleles for severely affected patients and in almost all of the alleles associated with the late-onset forms of 21-OH deficiency [5–9]. These point mutations are probably products of gene conversion events between the pseudogene and the CYP21B gene, and screening for a limited number of mutations allows the characterization of most of the mutated alleles, including de novo mutations [6]. We have recently reported the distribution and frequency of CYP21B mutations in the 21-OH-deficient Spanish population [9].

Allele-specific oligonucleotide (ASO) hybridization methods, based on isotopic labeling, are used to detect these mutations [2, 4, 7–11]. Highly sensitive isotopic methods have the disadvantage of the short half-life of labeled oligonucleotides and are not feasible in many clinical laboratories, given that special installations are needed. We have designed a protocol for the nonradioactive detection of point mutations in the 21-OH gene.

This nonisotopic protocol was applied to 15 families with 21-OH-deficient family members—5 with classical (CL) and 10 with late-onset (LO) forms. We also used a protocol involving radiolabeling to study 38 21-OH-deficient families (25 CL and 13 LO, some reported previously [9]). Five families were studied with both methods. Genomic DNA was isolated from peripheral blood leukocytes. Oligonucleotides were synthesized on an Applied Biosystems (Foster City, CA) 391 DNA synthesizer. PCR amplifications were carried out with a Pharmacia-LKB (Uppsala, Sweden) Gene ATAT thermal controller. The CYP21B gene was amplified in three overlapping fragments: exon 1 to exon 3 (fragment a), exon 3 to the noncoding 3′ termini (b), and exon 2 to exon 6 (c) as described previously [9]. Exons 3 and 6 are the regions selected for specific amplification of the gene [7–11]. The denatured PCR-amplified fragments