

Interference of Plant Peroxidases with Guaiac-based Fecal Occult Blood Tests Is Avoidable

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Peroxidase-rich fruits and vegetables are reputed to interfere with guaiac-based fecal occult blood tests. We added horseradish peroxidase to fecal samples and tested them with Hemocult[®], Hemocult SENSE[®], and hydrated Hemocult. Positivity rates with Hemocult and Hemocult SENSE decreased rapidly as the time between smearing (preparation) and development increased, whereas they remained high with hydrated Hemocult. For samples with added blood, positivity rates did not decrease with time. When 61 volunteers were tested on a standard restriction and on a challenge diet high in plant peroxidase, no positive results occurred during standard restriction. During the challenge diet, one volunteer was positive with Hemocult and Hemocult SENSE when development was delayed 24 h, and no volunteers were positive when it was delayed 48 h and 72 h. However, with hydrated Hemocult, positives occurred in 13 of 61 volunteers at 24 h, 8 of 61 at 48 h, and 5 of 61 at 72 h. Thus, peroxidase-rich plant foods do not need to be excluded from the diet with Hemocult and Hemocult SENSE if development is delayed for at least 48 h after smearing. A delay of this duration will not solve the problem of plant peroxidase interference with hydrated Hemocult.

Guaiac-based fecal occult blood tests (FOBTs)¹ are based on the detection of the peroxidase activity of heme/

hemoglobin (Hb) (1). As a consequence, ingestion of substances containing peroxidase activity is a potential source of false-positive test reactions (1, 2). Patients are, therefore, advised to abstain from certain peroxidase-rich foods for 72 h before and during sample collection for the test (2). Because this requirement is likely to adversely affect participation in testing, any simplification of the dietary requirements should prove beneficial.

Animal food products high in heme content (e.g., beef, lamb, and processed foods containing these meats) and certain raw peroxidase-rich fruits and vegetables (e.g., broccoli, cauliflower, radishes, turnips, and some melons) (3) are restricted during sample collection (2). Although good evidence exists to support the exclusion of animal food products with high heme content (4), no direct evidence exists for the exclusion of raw, peroxidase-rich fruits and vegetables.

Plant peroxidases, like Hb, are hemoproteins that have the prosthetic group ferriprotoporphyryn IX (hemin) (5). Plant peroxidases are thought to have a much higher enzymatic activity than Hb (6) because of differences in the apoproteins of the two molecules (7). The Hb apoprotein does not survive transit through the gut (8, 9), and a similar result might be expected for plant peroxidases. However, plant peroxidases are located within an indigestible cellulose cell wall (10), which should provide them with some protection against digestion. Their chances of surviving transit are probably further enhanced by plant peroxidase-associated glycans, which have been shown to protect them from proteolytic degradation (11).

The test reaction of chemical FOBTs takes place in a mixture of feces, guaiaconic acid, and an ethanol-based, hydrogen peroxide-containing developer. In theory, the greater the ethanol concentration of this environment, the more likely it is that the protein portion of the plant peroxidase will be denatured and, hence, the less likely that any plant peroxidases that survive digestion would influence the test result. Because the ethanol concentration of the reaction mixture is dependent on the ethanol concentration of the developer and the water content of the fecal smear, plant peroxidase interference should decrease as the fecal smear dries.

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¹Nonstandard abbreviations: FOBT, fecal occult blood test; Hb, hemoglobin; HO, Hemocult; HOS, Hemocult SENSE; HHO, hydrated Hemocult; HPE, horseradish peroxidase extract; and CI, confidence interval.

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This study was designed to investigate whether plant peroxidases are capable of interfering with the guaiac-based FOBTs, Hemocult® (HO), Hemocult SENSE® (HOS), and hydrated Hemocult (HHO) and, if interference does occur, whether it decreases as the time between fecal smearing and development increases.

Materials and Methods

FECAL OCCULT BLOOD TESTING

HO and HOS tests (SmithKline Diagnostics, Inc., Palo Alto, CA) were performed according to the manufacturer's instructions (12). The HHO test method was the same as the standard HO test method except that 25 μ L of water was applied to each fecal smear 1 min before addition of developer. A test was considered positive if any trace of blue appeared on or emanated away from the fecal smear within 1 min after the addition of developer.

All test cards were stored at room temperature away from light in a manner allowing them to air dry between the times of fecal smearing and development.

IN VITRO STUDIES

Single fecal samples were obtained from four healthy young subjects after each had completed a minimum of 3 days on a standard low heme/low plant peroxidase diet as described elsewhere (2). Before the protocol was begun, each fecal sample was tested and found to be negative for occult blood with each of the FOBTs used in the study.

Plant peroxidase was used in the form of crude horseradish peroxidase extract (HPE; ICN Biomedicals, cat. no. 195371) diluted to a working concentration of 10 g/L in distilled water. Hb was used in the form of whole blood collected in acid citrate-dextrose collection tubes.

HPE or whole blood was added to fecal samples 1 h after defecation. The concentrations of added HPE or whole blood were chosen to give low, medium, and high positivity rates for their respective analytes. The final concentrations of HPE were 0.3, 1.0, and 5.0 mg/g feces, and the final concentrations of Hb were 0.3, 0.5, and 1.0 mg/g feces. Test cards were smeared immediately after the addition of HPE or whole blood. For each FOBT, three test cards were prepared for each concentration of each fecal sample and developed at 1, 8, 24, 48, 72, and 144 h after smearing for both the plant peroxidase and Hb samples. Overall, 54 test cards (108 windows) were developed for each FOBT per fecal sample.

IN VIVO STUDIES

Healthy subjects, ages 18–30, with no history of bleeding hemorrhoids or personal or family history of relevant gastrointestinal disorders were recruited for the study. This group was chosen because it was highly unlikely that any subject would have a gastrointestinal disorder that would account for a positive FOBT. A total of 64 volunteers were enrolled in the study. None was taking non-steroidal antiinflammatory drugs. Each volunteer received written and oral information about the protocol and the purpose of the investigation and gave free and informed consent for inclusion in the study. The protocol

was approved by The Royal Melbourne Hospital Board of Medical Research (Project No. 136/94).

Three volunteers failed to comply with the dietary requirements of the study and withdrew. Of the 61 volunteers who completed the study, 32 were men and 29 were women. The mean age was 26 years.

The volunteers were tested on two separate dietary regimens with each of the three FOBTs: after being placed on a low heme/low plant peroxidase diet (diet A) and after a low heme/high plant peroxidase challenge diet (diet B). The diets were equivalent except that on the plant peroxidase challenge diet, the volunteers were provided with 750 g of raw peroxidase-rich fruits and vegetables, comprising 160–180 g of broccoli, 160–180 g of cauliflower, 40–50 g of red radish, 110–120 g of turnip, and 250–280 g of cantaloupe, to consume each day. They were permitted to eat any additional raw fruits and vegetables. The diets commenced 3 days before sample collection started and continued until sample collection was completed.

The volunteers prepared test cards, noting the day and time of passage, from each of three consecutive stools. For diet A, they prepared one test card (two windows) per FOBT per stool. For diet B, they prepared three test cards (six windows) per FOBT per stool. All samples were collected free from contamination by urine or toilet water, and all test cards were returned to the laboratory within 24 h after defecation.

Test cards for diet A were developed 24 h after defecation. For diet B, test cards were developed at 24, 48, and 72 h after defecation.

At the completion of diet B, the volunteers were provided with a dietary record and asked to estimate the percentage of each raw fruit and vegetable they actually consumed for each day of the diet. Reported percentages for the day on which the last sample was collected were excluded.

STATISTICS

Comparisons between HO, HOS, and HHO for samples with added HPE and Hb were made with the Kruskal–Wallis test (13). Where significance was found, pairwise comparisons of the three groups were made via the method of Dunn (14). Differences in positivity rates were compared by calculating the 95% confidence intervals (CIs) for the difference for paired cases (15).

Results

IN VITRO STUDIES

For the samples with added HPE, positivity rates for HO and HOS were initially high but decreased substantially as the time between sample preparation and development increased (Fig. 1). In contrast, for HHO the initial positivity rates were very high and remained high over the 6-day time course. HHO was significantly more affected by the added HPE than HOS at all time points and more affected than HO at all except the 1-h time point (Kruskal–Wallis test; $P = 0.02$ at 1 h, $P < 0.0001$ at 8 h, $P < 0.0001$ at 24 h, $P < 0.0001$ at 48 h, $P = 0.0003$ at 72 h, and $P = 0.001$ at 144 h).

For the samples with added whole blood, positivity rates for HO and HOS initially increased and then pla-

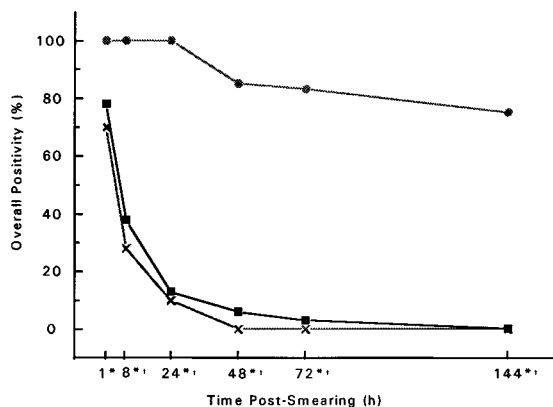


Fig. 1. Overall positivity rates of each FOBT for the fecal samples with added HPE in relation to time after smearing.

■, HO; X, HOS; ●, HHO; *, Kruskal-Wallis test, $P < 0.05$, pairwise difference between HOS and HHO; †, Kruskal-Wallis test, $P < 0.05$, pairwise difference between HO and HHO.

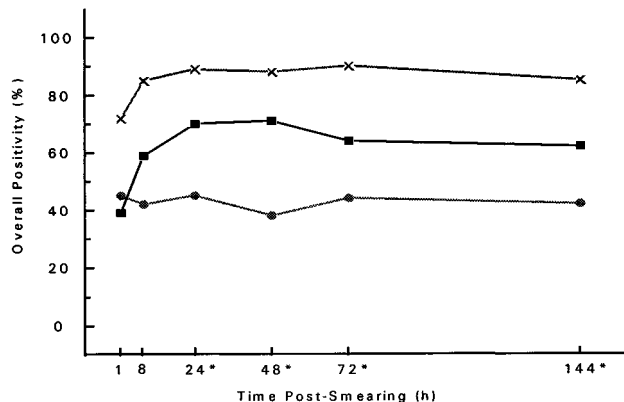


Fig. 2. Overall positivity rates of each FOBT for the fecal samples with added Hb in relation to time after smearing.

■, HO; X, HOS; ●, HHO; *, Kruskal-Wallis test, $P < 0.05$, pairwise difference between HOS and HHO.

teated, whereas the positivity rate for HHO remained constant (Fig. 2). HOS was the most sensitive test, with significant pairwise differences from HHO at the 24-h (Kruskal-Wallis test; $P = 0.03$), 48-h ($P = 0.03$), 72-h ($P = 0.03$), and 144-h ($P = 0.02$) time points. Despite the apparent greater sensitivity of HO over HHO, there were no significant pairwise differences between these two tests at any time point.

IN VIVO STUDY

Compliance. Given the very large quantity of fruit and vegetables the subjects were asked to consume, compliance was good. All of the supplied cantaloupe was consumed on 88% of study days, all broccoli was consumed on 83% of study days, all cauliflower was consumed on 83% of study days, all red radish was consumed on 82% of study days, and all turnip on 68% of study days.

FOBT results. No subject tested positive with any of the tests on the low heme/low plant peroxidase diet ($n = 61$). For the plant peroxidase challenge diet, the only positive HO and HOS test results occurred at 24 h after smearing, and only one volunteer tested positive with each of the tests (Fig. 3). There were no significant differences for either test when compared with their positivity rates on

the low heme/low plant peroxidase diet (differences for both the HO and HOS tests, 1.6%; 95% CI of the difference, -1.5% to 4.8%). For the HHO test, 13 volunteers were positive at 24 h, significantly more than on the low heme/low plant peroxidase diet (difference, 21.3%; 95% CI, 11.0–31.6%). Although the number of volunteers who tested positive with the HHO test decreased to eight at 48 h and then to five at 72 h, significantly more subjects still tested positive at each of these time points (48-h difference, 13.1%; 95% CI, 4.6–21.6%; 72-h difference, 8.2%; 95% CI, 1.3–15.1%). In addition, significantly more subjects tested positive with the HHO test at each time point on the high plant peroxidase challenge diet than with the HO or HOS tests (24-h difference, 19.6%; 95% CI, 9.7–29.6%; 48-h difference, 13.1%; 95% CI, 4.6–21.6%; 72-h difference, 8.2%; 95% CI, 1.3–15.1% for both HO and HOS).

Discussion

The results demonstrate that ingested plant peroxidases are capable of surviving transit through the gut and that, in the feces, they can cause positive guaiac-based FOBT reactions. For HO and HOS, they also show that the ability of plant peroxidases to cause false-positive reactions decreases rapidly as the time between fecal smearing and development increases. In contrast, positivity rates attributable to the peroxidase activity of Hb increase before plateauing over the same time course for these two tests.

Three previous studies were unable to demonstrate any interference by the ingestion of raw fruits and vegetables with HO (4, 16, 17), results that are compatible with those seen in the present study. There was, at best, only minimal interference with HHO in the one previous study that examined it (4), a result that differs from our present findings. However, the challenge diet in that study did not include the substantial amount of peroxidase-rich fruits and vegetables used in the present study.

Recently, Rozen et al. (18) postulated that the high HOS positivity rate observed in the Israeli colorectal cancer screening studies was caused by a culturally-based preference for peroxidase-rich fruits and vegetables over other

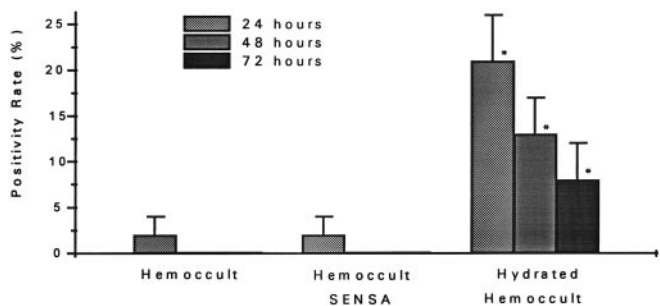


Fig. 3. Positivity rates of each FOBT for the 61 subjects on the low heme/high plant peroxidase challenge diet 24, 48, and 72 h after smearing.

The error bars represent the SE. *, significant at $\alpha = 0.05$ level compared with the low plant peroxidase diet.

food groups. They found that the positivity rate fell substantially when they delayed development until 3 days after sample application. In another study, they demonstrated that the HOS positivity rate obtained in subjects on a standard restriction diet was equivalent to that obtained in subjects on an unrestricted diet whose test cards were stored for at least 3 days before development (19). Their conclusions are compatible with the results obtained in this study.

Previously, we showed that HOS is capable of detecting lower concentrations of Hb in feces than HO (20, 21). This was observed as a nonsignificant trend in our study (Fig. 2). In this light, it is interesting that we found HOS to be no more affected by plant peroxidase interference than HO. This result may be explained by the fact that the HOS developer contains 5% more ethanol than the HO developer (12), consequently rendering a higher proportion of any plant peroxidases inactive because of denaturation.

Hydration of HO has been shown to decrease the detection limit of the test for blood (22–24) and to increase its clinical sensitivity for colonic neoplasia (24, 25). In this study, we found HHO to be, at best, no more sensitive for blood than HO. Because the fecal blood concentrations we looked at were lower than those examined in the previous studies, hydration may increase the sensitivity of HO only where higher concentrations of fecal blood are concerned.

HHO has been shown to have a lower specificity than HO (25). More recently, it was found to have a higher positivity rate and lower positive predictive value than HOS in a bowel cancer screening program without finding a substantially higher yield of lesions (26). The results of our study suggest that plant peroxidase interference may account, at least in part, for these observations. Overall, it appears that HOS has a distinct advantage over HHO in clinical practice because HOS is less prone to interference by plant peroxidases.

The key finding of our study was that a diet extremely high in raw peroxidase-rich fruits and vegetables does not affect HO or HOS if, after smearing, the test cards are stored at room temperature for 48 h or more before development. Therefore, raw fruits and vegetables may be eaten without restriction by subjects being tested for fecal occult blood by Hemoccult or Hemoccult SENSE, provided that test cards are stored at room temperature for at least 48 h before development. In contrast, stringent restriction of raw fruits and vegetables appears to be required for hydrated Hemoccult.

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