Series Editor: Melvin Schapiro, M.D.

Fecal Immunochemical Tests (FIT) vs. Office-Based Guaiac Fecal Occult Blood Test (FOBT)

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Fecal occult blood tests (FOBT) continue to have an important place in screening for colorectal cancer as they serve to identify people who are more likely to have neoplasia and so direct them to colonoscopy. There are two main FOBT technologies: guaiac or gFOBT and fecal immunochemical tests or FIT. They are quite different from each other in their biological, behavioural, clinical and technological characteristics. The criteria for the ideal FOBT are best met by FIT. With FIT, the whole sampling process is simplified for the individual, especially if the brush-sampling technology is used. Clinical performance is also better with FIT as they have a better sensitivity:specificity ratio. Ideally, sampling for FOBT are done at home at the convenience of the individual. In this setting, people are most willing to undertake FOBT of the brush-sampling FIT type.

INTRODUCTION

Fecal occult blood tests (FOBT) have a very real place in screening for colorectal cancer (CRC) (1). Their value is proven in randomized controlled trials at the population level. They meet WHO requirements (2) in that they are simple tests which serve to select out those with a higher probability of having CRC (3) to whom diagnostic, perhaps therapeutic, colonoscopy is then directed. Just as we use indicators of high risk to determine who gets surveillance colonoscopy, i.e. family history and past history of adenomas (3,4), the FOBT serves to profile risk. In fact, the person with a positive FOBT result is much more likely to have neoplasia than the person with a family history or past neoplasia.

It is important that a screening test, which is directed at healthy people, have an impact measurable at the population level (4). People are inherently reluctant to undergo invasive and inconvenient tests for screening such as colonoscopy without strong motiva-

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tion. Indeed, colonoscopic screening of everyone must be considered very carefully when a minority, only about 4%–7%, will develop CRC.

FECAL OCCULT BLOOD TEST (FOBT) TECHNOLOGIES

There is a range of types of tests for blood products in feces collectively referred to as FOBT (3–5). FOBT should be really qualified, however, to more accurately reflect the actual technology employed since they are by no means similar. The main commercial FOBT technologies detect either of two classes of hemoglobin product in feces. The guaiac FOBT (gFOBT) detect heme while fecal immunochemical tests (FIT) detect globin (Figure 1).

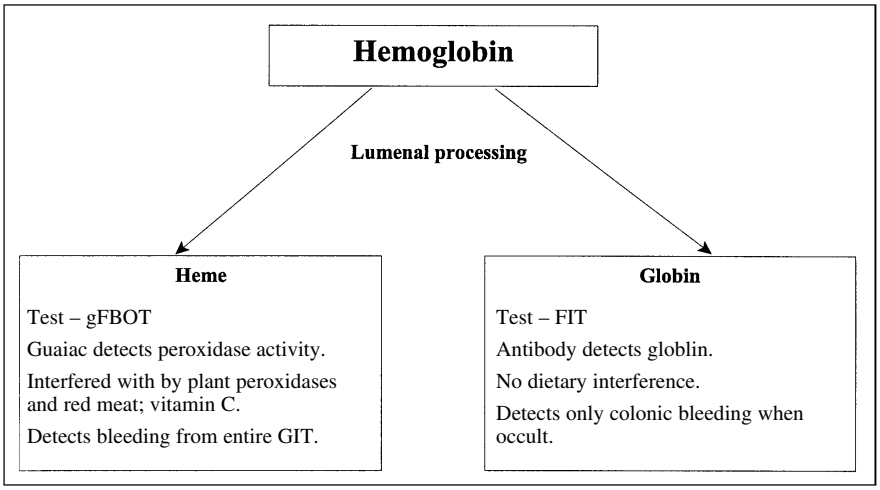

Figure 1. Schema for hemoglobin breakdown in feces and associated issues with the two FOBT technologies.

Detection of heme by gFOBT is dependent on the peroxidase activity of heme. Dietary peroxidases (found in a range of certain fruit and vegetables especially if raw) can cause false-positive results with gFOBT [see 3]. Antioxidants such as vitamin C may interfere with the chemistry of the reaction to cause false-negative results (see 4). Dietary heme from red meat also causes false positives (6). Heme is also reasonably stable in the gut and gFOBT may detect bleeding from any site in the GI tract although they are more sensitive for lower GI bleeding (3). This means that gFOBT are not selective for bleeding from colon/rectum.

Detection of globin is based on antibodies which are generally specific for human hemoglobin and some of its lumenally-derived degradation products (3-5). FITs are not subject to interference by diet or drugs and do not require proscription of any foods or drugs prior to sampling feces. As globin is rapidly digested in stomach and small intestine, FITs are much more selective for occult bleeding of colorectal origin than are gFOBT (3,5).

THE IDEAL FOBT

The primary use of gFOBT and FIT is in screening for colorectal cancer (CRC). They should therefore meet the requirements of WHO for tests used in population-based screening (2). Table 1 lists the ideal characteristics, considering what is involved in the screening process.

Ability to select out those most likely to have neoplasia is embodied in the pre-test/post-test likelihood ratio (3,4), or in more familiar terms, reflects the balance of sensitivity and specificity. Sensitivity facilitates detection of those with significant neoplasia while specificity effectively determines the number of colonoscopies needed.

Table 1
Characteristics of the ideal FOBT.

Sampling
- Convenient, without need to attend a physician.
- Readily organizable, without need for diet and drug restrictions.
- Acceptable, with easy and simple fecal sampling.

Performance
- Selective for colorectal bleeding.
- Able to select out those most likely to have neoplasia and to whom diagnostic colonoscopic resources are applied.

Tests development:
- Suitable for mass development of large numbers in a short time.
- Subject to ready quality control and objective reading of results with a stable, instrument-readable endpoint.

Table 2
Estimates of performance characteristics of different types of FOBTs.

Test and type	Specificity for neoplasia*	Sensitivity for cancer+
Rehydrated Hemoccult: gFOBT	90%	90+% with repeated annual screening.
Hemoccult II: gFOBT	94%–98%	35%–55% with once-off testing. Up to 80% with repeated annual testing.
HemoccultSENSA: gFOBT	88%-92%	80% with once-off testing.
Heme Select variants: FIT	95%	70%-82% with once-off testing.

Note: Adapted, updated and simplified from [whoguide].

OVERVIEW OF gFOBT PERFORMANCE

The role of gFOBT is clearly established in screening for CRC. A program of regular biennial screening with the Hemoccult II® gFOBT significantly reduces population mortality on an intention-to-screen basis by 15%–18% (7,8). Once-off sensitivity of Hemoccult II® is generally in the order of 35%–50% [see 9] although repeated annual screening increases sensitivity to 80% (10).

Several methods have been implemented in efforts to improve sensitivity. One is rehydration of fecal samples prior to development (10). Rehydrated Hemoccult II achieved a more substantial (33%) reduction in mortality from CRC (10) and also reduced incidence in the long term by 20% (11).

The performance characteristics of gFOBT are summarised in Table 2. In view of these trial results, many bodies have issued guidelines recommending that FOBT screening be undertaken, along with screening by other modalities (1,12).

While rehydrated Hemoccult II is more sensitive, it has poor specificity, caused by activation of plant peroxidases resulting from rehydration of fecal smears (13). It is therefore not recommended. Hemoccult II has been compared to rehydrated Hemoccult in two large studies: the Minnesota randomized controlled trial (10) and the Texas (MD Anderson Cancer Center) screening study (14). In the Minnesota study, the positivity rate of unhydrated Hemoccult was 2.4% and rehydration increased it to 9.8%. In the MD Anderson study, the positivity rates were 5% and 14.6% respectively.

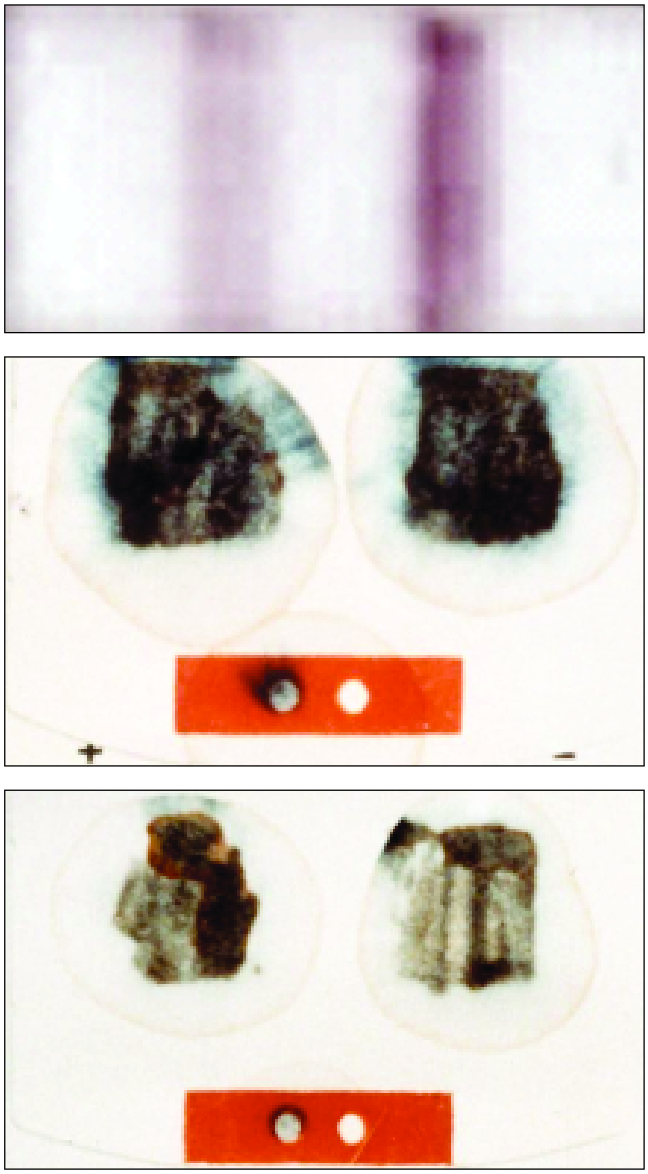

Figure 2. Examples of test endpoints for Hemoccult, Hemoccult Sensa and InSure FOBT. The latter is an FIT with a stable endpoint while the endpoint with the two gFOBTs is often transient.

In practice, dietary restriction can be confined to red meat alone by waiting three days before develop-

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Table 3
Shortcomings of guaiac-based FOBT (gFOBT)

- Restrictions on diet and drugs needed to optimize specificity and sensitivity, especially for the sensitive gFOBT.
- Generally use non-preferred spatula-sampling of stools with stool needing to be kept clear of toilet bowl water.
- Not selective for colorectal bleeding.
- Office-based testing might lead to increased false-positive results and suboptimal number of samples.
- Endpoint is transient and can be difficult to read.
- Sensitivity for cancer is suboptimal for less sensitive versions.
- More sensitive versions are subject to unpredictably high false-positive rates.
- Not suitable for mass-development or reading.

ing a guaiac test (15). One review suggested that restrictions were not needed (16) but this is not so for the more sensitive gFOBT (13). Restrictions of these interfering substances do need to be implemented three days prior to testing (6). Drugs such as aspirin may also cause false-positive results for neoplasia as aspirin can cause dose-related gastrointestinal bleeding (3).

With the Hemoccult Sensa® test, a new developer has enhanced sensitivity and improved readability and stability of the endpoint (17). An example of Hemoccult and Hemoccult Sensa test endpoints are shown in Figure 2. Care needs to be taken when reading tests and training and quality assurance is desirable (see 1). While Hemoccult Sensa® is not subject to interference by plant peroxidases provided that development is delayed for 72 hours after sampling (15) it is more affected by red meat ingestion than Hemoccult (13).

Comparison of Hemoccult Sensa® with Hemoccult II has been undertaken at five centers (see 9). Its positivity rate was always higher than Hemoccult II and generally twice as high. In some populations, e.g. California, there was an unacceptably low test specificity for HOSENSA (18) with a positivity rate of over six times that of Hemoccult while detecting twice as many cancers. Clearly, attempts to improve sensitivity with gFOBT lead to unpredictably variable and sometimes high positivity rates due to poor specificity.

Fecal sampling for gFOBT has commonly employed the traditional wooden spatula method when undertaken outside the doctor's office.

SHORTCOMINGS OF gFOBT

Even though of proven use, gFOBT are being used less often (1) for the reasons (Table 3) that they do not meet the criteria required of an ideal test (Table 1).

Use of gFOBT in the office setting is also a concern. Usually only one sample rather than the recommended three is obtained. The patient has rarely undertaken dietary preparation and so increases the risk of false-positives. There is always the concern that digital rectal examination will generate minor trauma and so lead to a positive result.

THE CHANGING FACE OF SCREENING

Screening for colorectal cancer (CRC) has several perspectives—that which applies to the individual and that which applies to the population (4). The imperatives for each are different.

The traditional mode has been a face-to-face meeting between the individual and a health professional—sometimes referred to as case-finding or individualistic screening. Here, the person can be assessed for symptoms and level of risk. What is offered is done in the context of counselling. Duty of care and what is best drives the decision-making—cost-effectiveness tends not to be a prime issue.

Population screening is becoming increasingly prominent. It seeks, through a common often impersonal approach, to engage individuals in at least some form of preventive activity—in effect, anything is better than nothing. Hence, if one seeks to have screening impact on CRC outcomes at the population level, simplicity, acceptability, feasibility and low initial cost with proven cost-effectiveness are needed. Many thousands will be tested in a short time-frame often without ability to ascertain presence of symptoms or to profile risk.

Even the approach of population screening is changing. Specificity itself has been a major consideration in the past (3), but now, as we see a trend to recommend screening by colonoscopy itself, specificity is seen as being less of an issue than sensitivity (1) and ability to detect advanced adenomas, not just cancer, is important.

OVERVIEW OF FIT CHARACTERISTICS

FITs appear well-placed to overcome the shortcomings of gFOBT and fit into this changing face of screening.

They have an inherent biological advantage with their selectivity for colorectal bleeding (3,5).

They are not subject to exogenous influences by diet and drugs and this provides a behavioural advantage for better particiaption. Removal of typical dietary restrictions for a guaiac test (see 3) can increase participation significantly in a country where red meat consumption is relatively high (19).

Stool-sampling processes have also evolved with FIT. The original wooden spatula used with early gFOBTs required multiple sampling from the surface of the stool which was ideally kept clear of the toilet bowl water. FITs have incorporated newer approaches. Some require a probe to be inserted into the stool (e.g. Bayer detect™ version of Fujirebio's Magstream® test) while others simply sample toilet bowl water from around the immersed stool (InSure®/InForm®, Enterix Inc.). Such new approaches may provide behavioural advantages if they are preferred over the older methods. But they also require validation as reliable means of achieving a representative sample. These points are further discussed below.

gFOBT are designed for small-scale in-office use; population screening requires rapid processing and development of many samples. With several commercially-available types, automated development is possible (e.g. Bayer detect™, Enterix's InSure). It is desirable to automate the reading of test end-points as well and this is also possible for several FIT. Some can give quantified endpoints although none is FDA-approved for this at present. Quantification facilitates standardization of methodology and maintenance of quality control. It also allows for adjusting the cut-off level for detecting fecal occult blood and deciding on who to colonoscope (3,20).

COMPARISON OF FIT WITH gFOBT

While FITs have not been compared to gFOBT in controlled trials of screening using CRC mortality as the end-point, several studies using informative surrogate end points have compared earlier commercially-configured FITs to several versions of gFOBT. This has been critically reviewed in detail and a full discussion is beyond the scope of this review (see 9).

HemeSelect® is a stick-sampling FIT that was originally developed as Immudia®HemSp by Fujirebio, (Tokyo, Japan) and has now evolved into the commercial tests Magtream HemSp and Bayer detect™. It has been extensively studied and shown in screening studies to detect more neoplasms than Hemoccult (17,18,21). Although it does not obviously appear to yield more neoplasms than HemoccultSensa, it provides an improved balance of sensitivity to specificity in that it is as sensitive but considerably more specific (18).

FlexSure OBT™ is a spatula-sampling FIT that has been accepted by FDA as a reference point (22) but it has not remained commercially available. A new brush-sampling FIT—InSure—compares well with it for sensitivity and specificity (22).

Overall, FIT with published data to support performance can be expected to have a better sensitivity:specificity balance than do gFOBT and so perform better in selecting out those who are more likely to have neoplasia.

BRUSH-SAMPLING FIT

Most FIT use a variation of stick-based sampling of the stool although in most instances this has evolved from the wooden spatula used with the commonest gFOBT to a simple probe that once used to sample the stool is placed into a plastic tube with preservative.

In an effort to develop a more acceptable and simpler sampling methodology, a brush-based sampling technique has been developed. The participant is asked to sample toilet bowl water from the surface of the immersed stool by swishing the brush in the bowl. This has been combined with an immunogold membrane test which uses a dual antibody system specific for human hemoglobin. The resultant InSure test (Enterix Inc., also known as InForm in Australia) provides an endpoint which is stable (Figure 2) and highly readable by eye as well as by optical technology that allows quantification (23). Sample card development can be done completely by robot.

Initial pre-screening evaluations of this brush-sampling FIT showed it to have similar specificity and sensitivity to the FIT *FlexSure OBT* (22).

In an evaluation of the acceptability of the brush-sampling methodology to the general population, three randomly selected cohorts in urban Adelaide were allocated to a mail offer of either Hemoccult (spatula-sam-

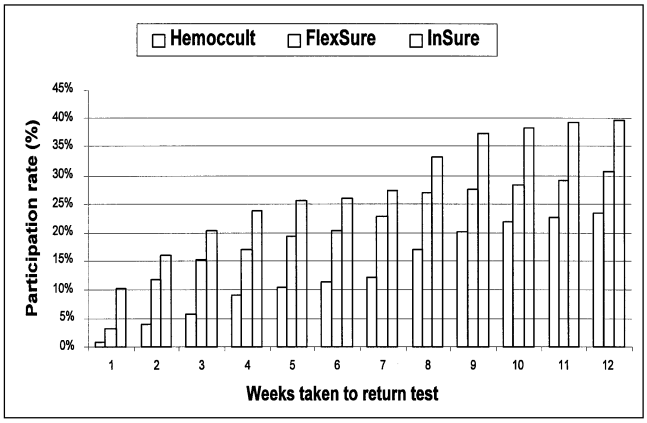

Figure 3. Effect of test technology on participatory behaviour in population screening for colorectal cancer (23).

pling of stool with diet restriction), *FlexSure OBT* (spatula-sampling of stool without diet restriction) or InSure (brush-sampling of stool without diet restriction) (23). As can be seen from Figure 3, population participation increased with removal of diet restrictions and further increased with introduction of the brush-sampling method. By 12 weeks, participation with InSure testing was almost double that of Hemoccult (40% vs 24%).

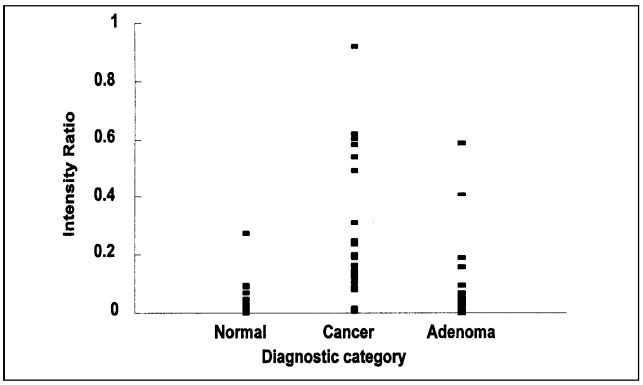

Figure 4. Fecal hemoglobin concentrations in patients with neoplasia.

Concentrations are expressed as intensity ratio relative to control line, measured using an adaptation of the InSure test where the endpoint is quantified by an optical device. Normal n = 166, cancer n = 22, adenoma n = 18. Each group is significantly different from the other, $p < 0.05$.

Quantification of the fecal immunochemical test strips was determined using a prototype machine reader and computer software developed by Larry Lapointe and Howard Chandler. After digital image acquisition and processing, each immunochromatographic result was quantified based on the ratio of the colloidal gold signal found at the hemoglobin test line to the corresponding signal of the internal control line. For quantification purposes, this ratio is referred to as the Intensity Ratio.

Because the sampling method is novel, it was evaluated for its ability to provide quantified results that differentiated between those who had cancers or adenomas and those who were normal at colonoscopy. A novel optical method for digital image acquisition and processing of the immunochromatographic result was specifically developed for this purpose (Larry La Pointe, Howard Chandler, personal communication). The results, shown in Figure 4, clearly show good differentiation between these three clinical groups. They also show that people with adenomas may bleed.

A direct within-individual comparison of InSure with Hemoccult Sensa is now underway and has been reported in abstract form (24). Two populations were asked to sample two stools using the brush-sampling technique of InSure and three with the Hemoccult device, prior to colonoscopy: a) Community screening (n = 443), all those positive by qualitative endpoint were colonoscoped; b) Colonoscopic examination for high risk settings (n = 202). Predetermined diagnostic categories were allocated independent of FOBT result. InSure was significantly more sensitive than Hemoccult Sensa, detecting 16/18 cancers compared to 9/18. It also detected significantly more adenomas 27/51 versus 18/51. False-positive rates were similar at 7.8% and 7.0% respectively. Expressed in another way, InSure resulted in 21 more colonoscopies being done than did Hemoccult SENSA but it detected 7 more with cancers and 9 more with adenomas.

AVAILABILITY OF FIT

On the international scene, FIT have been well accepted in terms of reimbursement and/or government-funded national programs in countries such as Australia and Japan. Other countries are planning to follow.

In the USA, the Centers for Medicare and Medicaid Services (CMS) have expanded the range of screening options covered under the Congressionally-mandated Medicare colorectal cancer screening benefit, to include annual screening using FIT. This paves the way for appropriate reimbursement.

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CONCLUSIONS

FOBT continue to have an important place in screening for colorectal cancer as they serve to identify people who are more likely to have neoplasia and so direct them to colonoscopy. There are two main FOBT technologies: guaiac-based and immunochemical-based. They are quite different from each other in their biological, behavioural, clinical and technological characteristics. The criteria for the ideal FOBT are best met by FIT. With FIT, the whole sampling process is simplified for the individual, especially if the brush-sampling technology is used. Clinical performance is also better with FIT as they have a better sensitivity:specificity ratio. In this setting, people are most willing to undertake FOBT of the brush-sampling FIT type. FIT should replace gFOBT as the simple and inexpensive approach to population-based screening for CRC. ■

References

1. Allison JE. Screening for colorectal cancer 2003: Is there still a role for the FOBT? *Tech Gastrointest Endosc,* 2003;5:127-133.
2. Watson JMG, Jungner G. Principles and Practice of Screening for Disease. *WHO Public Health Paper 34,* 1968.
3. Young GP, Macrae FA, St John DJB. Clinical methods for early detection: Basis, use and evaluation. In: Young GP, Rozen P, Levin B Eds. *Prevention and Early Detection of Colorectal Cancer,* London, Saunders, 1996: 241-270.
4. Young GP, Rozen P, Levin B. How should we screen for early colorectal neoplasia. In: Rozen P, Young GP, Levin B, Spann SJ. Eds. *Colorectal Cancer in Clinical Practice,* London, Martin Dunitz, 2002:77-99.
5. Young GP, St John DJB. Faecal occult blood tests, choice, usage and clinical applications. *Clin Biochem Rev,* 1992; 13:161-167.
6. Feinberg EJ, Steinberg WM, Banks BL, Henry JP. How long to abstain from eating red meat before fecal occult blood tests. *Ann Intern Med,* 1990; 113:403-404.
7. Hardcastle JD, Chamberlain JO, Robinson MHE, Moss SM, Amar SS, Balfour TW, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet,* 1996; 348:1472-1477.
8. Kronborg O, Fenger C, Olsen J, Jorgenson OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet,* 1996; 348:1467-1471.
9. Young GP, St John DJB, Winawer SJ, Rozen P. Choice of fecal occult blood tests for colorectal cancer screening: recommendations based on performance characteristics in population studies. *Am J Gastroenterol,* 2002;97:2499-2507.
10. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. *N Engl J Med,* 1993; 328:1365-1371.
11. Mandel JS, Church TR, Bond JH, Ederer F, Geisser MS, Mongin SJ, et al. The effect of fecal occult-blood screening on the incidence of colorectal cancer. *N Engl J Med,* 2000; 343;1603-1607.
12. Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, Mulrow CD, et al. Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology,* 1997; 112:594-642.
13. Sinatra M, St John DJB, Young GP. Interference of plant peroxidases with guaiac-based fecal occult blood tests is avoidable. *Clin Chem,* 1999, 45:123-126.
14. Levin B, Hess K, Johnson C. Screening for colorectal cancer: a comparison of 3 fecal occult blood tests. *Arch Intern Med,* 1997; 157:970-976.
15. Rozen, P, Knaani J, Samuel Z: Eliminating the need for dietary restrictions when using a sensitive guaiac fecal occult blood test. *Dig Dis Sci,* 1999; 44:756-760.
16. Pignone M, Campbell MK, Phillips C. Meta-analysis of dietary restriction during fecal occult blood testing. *Eff Clin Pract,* 2001;4:150-156.
17. St John DJB, Young GP, Alexeyeff MA, Deacon MC, Cuthbertson AM, Macrae FA, et al. Evaluation of new occult blood tests for detection of colorectal neoplasia. *Gastroenterology,* 1993; 104:1661-1668.
18. Allison JE, Tekawa IS, Ransom LJ, Adrian AL. A comparison of fecal-occult blood tests for colorectal-cancer screening. *N Engl J Med,* 1996; 334:155-159.
19. Cole SR, Young GP. Participation in faecal occult blood test-based screening for colorectal cancer is reduced by dietary restriction. *Med J Aust,* 2001;175:195-198.
20. Castiglione G, Grazzini G, Miccinesi G, Rubeca T, Sani C, Turco P, Zappa M. Basic variables at different positivity thresholds of a quantitative immunochemical test for faecal occult blood. *J Med Screen,* 2002;9:99-103.
21. Zappa M, Castiglione G, Paci E, Grazzini G, Rubeca T, Turco P, Crocetti E, Ciatto S. Measuring interval cancers in population-based screening using different assays of fecal occult blood testing: the District of Florence experience. *Int J Cancer,* 2001;92:151-154.
22. Young GP, St John DJB, Cole SR, Bielecki BE, Pizzey C, Sinatra MA, Cadd B, Morcom J. A prescreening evaluation of a brush-based faecal immunochemical test for haemoglobin. *J Med Screen,* 2003; 10: 123-128.
23. Cole SR, Young GP, Esterman A, Cadd B, Morcom J. A randomized trial of the impact of new fecal hemoglobin test technologies on population participation in screening for colorectal cancer. *J Med Screen,* 2003; 10: 117-122.
24. Cole S, Smith A, Bampton P, Sandford J, Morcom J, Young GP. Screening for Colorectal Cancer: Direct Comparison of a Brush-Sampling Fecal Immunochemical Test for Hemoglobin with Hemoccult. *Gastroenterology,* 2003;124:A80.