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Screening for Colorectal Cancer by Immunochemical Fecal Occult Blood Testing

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Screening for colorectal cancer using the conventional Hemoccult test has been shown to reduce mortality associated with cancer by 33% through a randomized controlled trial. However, the magnitude of effectiveness is small in terms of cost-effectiveness. The recently developed immunochemical fecal occult blood test (IFOBT) provides a potential replacement for the Hemoccult test as a screening test, due to its superior performance characteristics such as higher sensitivity shown in preliminary studies and the fact that it does not require any dietary restriction. The IFOBT method is reviewed, especially in relation to its specificity. In known colorectal cancer subjects, IFOBTs have shown both higher sensitivity and specificity than the Hemoccult test. Similarly, IFOBT has demonstrated a higher sensitivity than Hemoccult for colorectal cancer in an asymptomatic population. A nationwide screening program in Japan has demonstrated the feasibility of this approach for large population screening. However, the positivity rate varied according to the conditions at each screening facility. Therefore, technical factors that influence the positivity rate of IFOBTs in the screening program are discussed. Case-control studies have strongly suggested that screening using IFOBT would reduce mortality from colorectal cancer by 60% or more. Several observational studies have provided support for this estimate. The feasibility and effectiveness of population-based screening by IFOBT are discussed.

Key words: Colorectal cancer screening — Immunochemical fecal occult blood test — Immunochemical hemagglutination test — Sensitivity — Mortality reduction

Introduction

The ultimate goal of screening for colorectal cancer is to accomplish a reduction in mortality, which has recently been increasing. Colorectal cancer is a major cause of cancer deaths many countries.[1] Colorectal cancer mortality and incidence have also shown in a marked increase in Japan.[2] Age-adjusted mortality from colorectal cancer has doubled during the past two decades, from 5.2 and 5.0 per 100,000 population of males and females, respectively, in 1970 to 12.9 and 9.4 per 100,000 population in 1990.[2] Thus, secondary prevention of colorectal cancer is clearly important.

Fecal occult blood screening for colorectal cancer has been a potentially effective screening strategy since the appearance of the guaiac-impregnated slide test, or Hemoccult test, in the late 1960s.[3] Since there was no scientific evidence of effectiveness or mortality reduction by such screening, occult blood screening for colorectal cancer was not recommended as a public health policy measure,[4] except in West Germany in the late 1970s.[5] In 1993, annual occult blood screening for colorectal cancer was finally shown to reduce cancer mortality by 33%.[6] However, a new test is needed, because the Hemoccult test is not cost-effective and requires burdensome dietary restriction.[7–9] Theoretically, an immunochemical fecal occult blood test (IFOBT) can overcome these disadvantages. IFOBT by reversed passive hemagglutination reaction (RPHA)[10] has been shown to be superior to the Hemoccult test both in terms of sensitivity and specificity.[11–13] Subsequently, several IFOBTs have become commercially available. Most of these tests have demonstrated almost equal sensitivity and specificity to the original RPHA test. Consequently, a screening program using IFOBT was adopted as a public health policy in Japan in 1992 based on the potential efficacy of the screening suggested by a case-control study conducted in areas where screening had once been performed using the Hemoccult test, and where this had subsequently been replaced by IFOBTs.[11, 14] Later, a screening program using the RPHA test alone was evaluated more precisely, and was calculated to reduce colorectal cancer mortality by 60%.[15]

Today, the Japanese screening program for colorectal cancer seems promising; however, several problems must be overcome in order to achieve greater effectiveness using the program.[11] In the present paper, methods of IFOBT and the effectiveness of screening programs that use IFOBT are reviewed, based primarily on studies using the RPHA test, because screening results and program evaluations for other testing methods have not been fully reported.

Methods of immunochemical fecal occult blood testing

The immunochemical detection of human blood in feces was first described by Adams in 1974.[16] The report suggested that identification of the site of bleeding in the gastrointestinal tract might be possible by using antisera raised against human hemoglobin (Hb) and stroma of erythrocytes.[16] Subsequently, Barrows *et al.* reported a single radial immunodiffusion method,[17] which was later simplified by using a punched smear-disc.[18] Songster first suggested IFOBT for detecting colorectal cancer using the single radial immunodiffusion test, showing that the test yielded positive test results in 65% of 150 colorectal cancer patients, compared with only 40% by the Hemoccult test.[18] Following these studies, several tests based on immunochemical methods have been examined.[19–25] Of these tests, several were shown to have superior sensitivity and specificity to the conventional guaiac test in specimens from known colorectal cancer patients and normal controls.[18, 21, 23, 24] However, large-scale screening using these tests was not feasible, due to their complex and time-consuming procedures. Furthermore, a small population screening trial using an enzyme-linked immunosorbent assay (EIA) yielded disappointing results with a high false-positive rate,[26] although the immunochemical test should have been successful in terms of specificity. Specificity and sensitivity generally show an inverse relationship ("trade-off").[27] Even a test with 100% sensitivity may not be a worthwhile screening method if its specificity is too low.[28] Concerning colorectal cancer screening, the balance should preferably favor specificity due to patients' unwillingness to accept diagnostic investigation of the colon.[28] Moreover, the capacity to perform such examination in daily clinical practice is limited.[11] Therefore, determining the requirements for achieving high specificity of IFOBT has been extremely important, as a suitable test that yields results superior to those of the Hemoccult test in a large population screening.

Several factors that influence the specificity of IFOBTs were identified.[21, 29] These factors, and the method required to achieve high specificity of IFOBTs, are described below, together with the method for preparing and performing the immunochemical hemagglutination test or RPHA test.[10] The latter was developed based on specificity and sensitivity data and is considered to be applicable for population screening.

1. Preparation of anti-Hb antibody and factors that influence specificity

Hb and its derivatives excreted in feces

Hb is degraded into derivatives such as heme and heme-derived porphyrins after entering the gastrointestinal tract.[30, 31] Heme is released from globin and degraded into heme-derived porphyrins. Globin is degraded by proteolysis, thereby losing its immunoreactivity during transit. Globin is less resistant than heme to degradation in the gut.[32] The degree of Hb conversion to its derivatives depends mainly on the bleeding site, as well as on the amount of blood and the transit time.[33, 34] Thus, feces contain a mixture of intact Hb, free heme, and several kinds of heme-derived porphyrins in a proportion that depends on the bleeding site. The more proximal the bleeding, the less likely is intact Hb to be present in the feces, and *vice versa*.[32] Studies on the fate of Hb ingested by healthy volunteers have shown that almost no intact immunoreactive Hb is recovered in feces, whereas heme and porphyrins are easily detected.[31, 32] Because IFOBTs only detect immunoreactive Hb and globin, including an early-degradation form of globin,[32, 35] they can be considered to detect specifically lower gastrointestinal bleeding.[31, 32]

Specificity of anti-Hb antibody

Although a fundamental requirement of any immunochemical method is to use a highly specific antibody against the antigen to be targeted, little has been reported concerning basic specificity properties of anti-Hb antibody for IFOB testing. Two conditions are required for anti-Hb antibody in order to achieve high specificity in fecal occult blood testing.[29]

Anti-Hb antisera raised against crude or insufficiently purified hemoglobin can react with fecal extract from healthy persons without intestinal lesions.[21, 29] This false-positive reaction by anti-crude Hb occurred more frequently than that with the Hemoccult test in normal controls.[21] It has been shown to result from a contaminant antibody to non-Hb protein, carbonic anhydrase, that is present in erythrocytes and feces.[21, 29] Human colonic epithelial cells have been demonstrated to contain large amounts of carbonic anhydrase isoenzymes.[36] The antisera raised against purified Hb A_0 does not cause such false-positive results.[29]

Another problem relating to the specificity of anti-Hb antiserum is cross-reactivity of the serum with animal Hbs. Even antiserum raised against highly purified human Hb A_0 that appears to be specific for human Hb in immuno-precipitation experiments reacts with several species of animal Hbs when a sensitive method such as EIA is used to detect Hb.[29] This cross-reactivity is thought to be dependent on the homology of the amino acid sequences of antigenic sites in the globin moieties between human Hb and animal Hbs.[29, 37] Since the anti-human Hb A_0 antibody population consists of several subfractions, such as anti-α chain of globin, anti-β chain of globin, anti-$\alpha\beta$, and anti-$\alpha_2\beta_2$, cross-reactivity can result from any subfraction that has affinity for animal globins.[38] A small subfraction bound to chicken Hb was observed in the IgG fraction of anti-Hb A_0 antiserum. This fraction also reacts with other animal Hbs.[29] Such

cross-reactivity is removed after further purification by adsorption of the serum on a chicken Hb-Sepharose 4B column, except that with monkey Hb.[29]

2. Other factors that influence specificity and sensitivity

Optimal cut-off point for fecal Hb value

The existence of a small amount of Hb in stools from normal subjects has been demonstrated using the ^{51}Cr-tagged erythrocyte technique, which quantitates gastrointestinal blood loss by measuring radioactivity in stools. This finding raises the possibility that such Hb might cause a false-positive result in highly sensitive IFOBT.[29] Physiological intestinal blood loss was estimated to be 0.5–2.0 ml/day, or 0.5–2.0 mg/g stool assuming that 150 g of stools is passed per day and that the normal blood Hb value is 15 g/dl.[39–42]

Knowing the physiological concentration of fecal Hb is very important for determining a suitable cut-off point for an IFOBT,[29, 32] but few studies have investigated the amount of intact Hb in feces. Fecal immunoreactive Hb in controls was estimated to be less than 0.5 mg/g stool by IFOBT employing counter immunoelectrophoresis.[24] This value was determined to be 0.1–0.2 mg/g stool using the RPHA method. Fecal Hb and its derivatives were quantitated to be 0.72 mg/g[43] or 0.32 ± 0.09 mg/g[33] in normal subjects, of which an average of nearly 50% was porphyrin converted from heme. Threshold values for fecal Hb in other IFOBTs are 0.3 mg/g stool[18] and 0.7 mg/g stool[23] in single radial immunodiffusion and EIA, respectively. Although these values are slightly lower than those measured by radioassay, the discrepancy is not surprising. The normal range of Hb measured by IFOBT reflects the level of intact Hb and immunoreactive derivatives, and is lower than that measured by radioassay, which reflects total radioactivity in stools.[24, 32]

The other important determinants of the optimal cut-off point are distributions of fecal Hb values in subjects with colorectal cancer and those with several benign conditions. As described above, bleeding proximal to the colon does not generally present immunoreactive Hb in stool unless the bleeding is massive.[33, 34] Hence, bleeding from colonic lesions must be considered in determining the cut-off point for IFOBT. Most clinically diagnosed colorectal cancers present higher fecal Hb values than those of controls,[10, 23] although the amount of bleeding from the cancer lesion varies from day to day. Fecal Hb values measured by RPHA testing tended to be more than 8 times higher in 60% of colorectal cancer subjects as compared to the maximum value in controls.[10] The mean fecal Hb values measured using the EIA method were reported to be 11.3 mg/g stool and 0.4 mg/g stool in colorectal cancer subjects and controls, respectively.[23] Other studies have also shown larger amounts of blood loss in colorectal cancer subjects than in controls.[43, 44] However, there is overlap in the range of blood loss between colorectal cancer patients and control subjects.[10, 44] As a result, FOBT cannot completely discriminate between cancer-bearing and normal subjects. The frequency of bleeding from colon polyps is believed to depend on the size of the polyp. Polyps with a diameter of 2 cm or larger tend to bleed more often than those with a diameter of less than 1 cm.[45] This information is advantageous for screening in order to avoid unnecessary diagnostic investigations, because subjects with polyps that have a diameter of less than 1 cm need not be included in the high-risk group.[46, 47] Fecal blood levels measured using heme-porphyrin assays in subjects with diverticula and hemorrhoids are reported to be identical to those in patients without gastrointestinal lesions.[43] Therefore, the cut-off point should be determined after considering both the sensitivity and specificity associated with specimens from colorectal cancer subjects and controls.

Sample reproducibility

The amount of stool provided by the subject is also an important factor affecting the sensitivity and specificity of IFOBT.[29] A large sample from a normal subject may contain a sufficient quantity of physiological Hb to cause a false-positive reaction. Furthermore, the proportions of solid and liquid components vary among specimens with different consistencies.[29] Thickly-smeared stool is several times heavier than thinly-smeared stool. Fecal consistency determines the thickness of the smear.[29] Such variation among specimens influences test results. Consequently, a sampling device that facilitates preparation of a reasonably constant amount of feces is necessary. Although no ideal method for sampling a precise quantity of specimen is available, a filter paper card on which stool is smeared provides a relatively constant sample quantity.[29]

Stability of Hb in feces

Although IFOBT can detect partially degraded globin, fecal Hb rapidly loses its antigenicity at room temperature,[29] probably due to the large amount of bacteria in feces.[31] This change is dependent on the temperature at which the specimen is stored, suggesting enzymatic degradation.[29] Therefore, IFOB testing should preferably be performed immediately after preparing a specimen; otherwise storing a specimen at a maximum of 4°C before testing is recommended. Preservatives are not effective for preventing the degradation of Hb after preparing the specimen.[29]

Interpretation of test results

Besides sample reproducibility, incorrect interpretation of test results such as pattern changes in hemagglutination and latex agglutination can cause false-positive or false-negative results, owing to observer error.[32] In order to improve the accuracy of test interpretation, training is necessary, even for simple Hemoccult testing and especially for "borderline" interpretation of

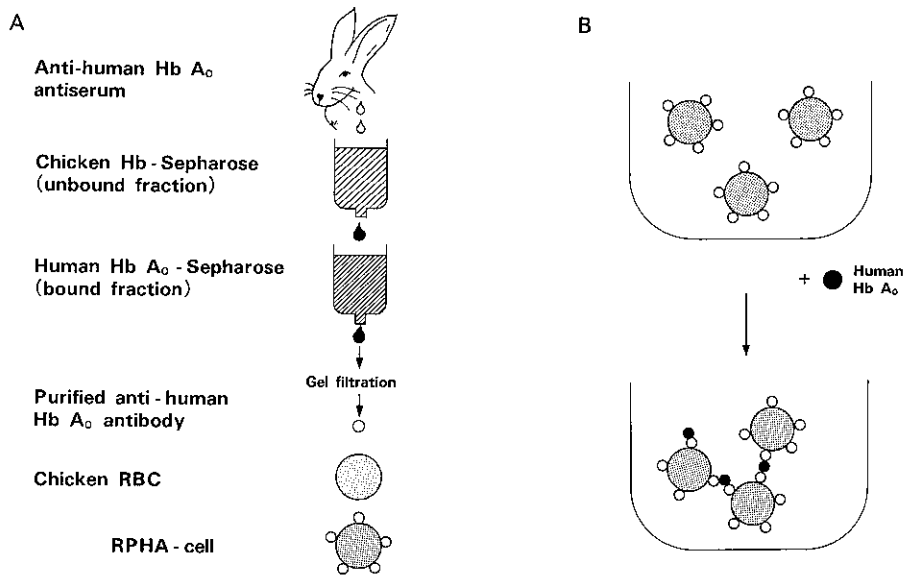

Fig. 1. A, Procedure for preparing anti-Hb A_0 antibody-coated chicken erythrocytes. B, Scheme of agglutination of antibody-coated cells (RPHA-cells) with human Hb A_0.

test results.[48] Training is also necessary for visual IFOBT interpretation.

3. Immunochemical hemagglutination (RPHA) test

Preparation of the RPHA-cells

Based on findings related to basic issues such as the specificity of the anti-Hb antibody, we established the RPHA test, which is applicable to large population trials.[10] The procedure for preparing anti-Hb A_0 antibody-coated cells for the testing and that for the RPHA reaction are shown in Fig. 1. Rabbit anti-Hb A_0 antiserum is purified by 2-step affinity chromatography and gel filtration in order to obtain a highly specific antibody to human Hb A_0, which is the major component of adult Hb. The purified anti-Hb A_0 was coated with formalinized, tanned chicken erythrocytes (RPHA-cells) (Fig. 1A). When Hb exists in fecal extract from the stool-smeared filter paper, the cells show agglutination (Fig. 1B). Cells prepared in this manner do not react with animal Hbs, except those of monkey, or with animal myoglobin or hemocyanin.[29]

Test procedure

The test procedure consists of Hb extraction from the feces-smeared filter paper disc, serial 1 : 2 dilution of the extract, and reaction of the cells with the diluted extracts. Two hundred μl of phosphate-buffered saline (10 m*M* sodium phosphate buffer, pH 7.2) was poured into the first (well 1 in Fig. 2) and second (2) wells of each row. Two filter paper discs removed from different parts of the fecal smear were soaked in the first and second wells.

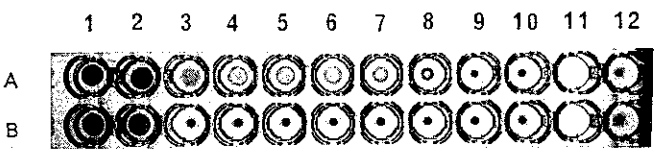

Fig. 2. Hemoglobin titration in fecal extract with anti-Hb A_0 antibody-coated chicken erythrocytes (RPHA-cells). The top row (A) shows a colon cancer specimen with agglutination in the 3rd to 8th wells, judged as 2^5. Interpretation of the end-point at the 8th well might be difficult for an unskilled person. The bottom row (B) shows a specimen from a normal control with a negative button through the wells, judged as $< 2^0$. The final well (12) of each row contains the original extract and uncoated cells. The negative button in each well clearly indicates the absence of non-specific agglutination in the extract.

Fecal extraction was performed by repeatedly pipetting phosphate-buffered saline in order to elute fecal Hb in the first and second wells. Each (1 or 2) extract was mixed in the third well (3, original extract, 2^0 dilution), and then doubly diluted serially with phosphate-buffered saline in the remaining wells (4–10, 2^1–2^7 dilution). Twenty-five μl of RPHA-cells was added to perform the RPHA reaction. Fecal Hb is titrated semiquantitatively (such as 2^1 or 2^6), based on the highest dilution that shows agglutination in the microplate (Fig. 2).

Table I. Hemoglobin Titration in Stools from Normal Controls with the RPHA Test

		RPHA titer[a]					
		n	$\leq 2^1$	2^2	2^3	2^4	$2^5 \leq$
Control	1[b]	36	35	1	0	0	0
Control	2[c]	567	542	21	3[d]	1	0

RPHA: Immunohemical fecal occult blood test by reversed passive hemagglutination reaction.
a) Highest dilution showing agglutination by reversed passive hemagglutination reaction.
b) Thirty-six specimens from 33 patients without gastrointestinal lesions confirmed by upper gastrointestinal endoscopy and colonoscopy.
c) Five hundred and sixty-seven specimens from 567 healthy, asymptomatic volunteers without detectable lesions by rectal digital examination. Subjects whose specimens showed a titer of 2^2 or more underwent colonoscopy and gastric endoscopy.
d) Including one patient with gastric cancer and another with colon polyp measuring 11 mm in diameter.

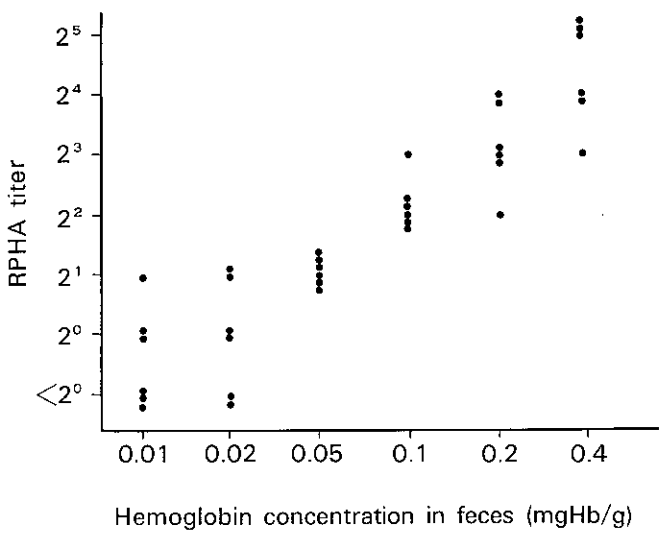

Fig. 3. Correlation between RPHA titer and hemoglobin concentration in mixture of feces and hemoglobin. Stool specimens from 6 normal controls without gastrointestinal lesions, in which the RPHA titers were $< 2^0$, were homogenized. To 1 g of each specimen, 0.5 ml of hemolysate containing 0.02–0.8 mgHb/ml was added to prepare 0.01–0.4 mgHb/g stool. RPHA titer is correlated with fecal hemoglobin concentration. Titer 2^2, the maximum titer in normal control (see Table I), is equivalent to 0.1–0.2 mgHb/g stool.

Determination of cut-off point

The optimal cut-off point was determined to be at 2^2 dilution of the original extract based on the distribution of titers among specimens obtained from 33 controls without gastrointestinal lesions and confirmed by endoscopy (Table I). Furthermore, from the results of 567 controls without rectal lesions, specificities were calculated to be 96% and 99% for the cut off titers of 2^1 and 2^2, respectively. Thus, the cut-off point of 2^2 was confirmed to be appropriate (Table I). A titer of 2^2 corresponds to 0.1–0.2 mg Hb/g stool (Fig. 3).

After the test was proven to have higher sensitivity and specificity than the Hemoccult test for outpatient screening in known colorectal cancer subjects, an initial population screening program was conducted in 1983.[11]

Application of IFOBT to screening program for colorectal cancer

Screening tests must have high sensitivity, specificity, and positive predictive value,[1, 28] should be easy to perform, and should be acceptable to subjects in order to be applicable to large-scale population screening and thereby lead to a reduction in mortality. As a feasibility indicator, the compliance rate is important, in addition to good performance characteristics.[28]

1. Comparison of efficacy between immunochemical tests and conventional chemical tests in known colorectal cancer subjects and controls

The present author developed the RPHA test and showed that the test has higher sensitivity and specificity than conventional chemical tests in specimens obtained from colorectal cancer subjects.[10] St. John *et al.* reported that the test showed 97% sensitivity in 107 patients with colorectal cancer, whereas the Hemoccult and HemoQuant test, which is a heme-porphyrin assay test, showed only 89% and 71% sensitivity, respectively.[12] The RPHA test also demonstrated a sensitivity of 76% in 45 subjects with adenomas larger than 1 cm in diameter, whereas Hemoccult and HemoQuant showed 42% sensitivity.[12] After the efficacy of the RPHA test was demonstrated, several immunochemical test kits were developed and became commercially available.[11] The sensitivity and specificity of these test kits and conventional chemical tests were compared using receiver-operating characteristic curves (ROC).[49] As a result, the superiority of immunochemical methods over conventional tests was shown, although the sensitivity and specificity differed among the immunochemical test kits.[11, 49] The sensitivity of IFOBT is as low as approximately 50% for early cancer lesions measuring less than 1 cm in diameter.[49]

2. Sensitivity and specificity of preclinical colorectal cancer evaluated in asymptomatic population

Implication of sensitivity according to estimation method

The sensitivity of IFOBT was shown to be higher than that of chemical tests in stool specimens from known colorectal cancer subjects, as described above.[11, 12, 18, 24]

However, these figures do not represent actual sensitivities in a screening setting, because they are based on results for specimens obtained primarily from symptomatic subjects.[27, 50] Calculation of sensitivity using symptomatic colorectal cancer patients is useful for comparing performance characteristics among the tests. In an asymptomatic population, the usual target of the screening program is cancer in the preclinical stage.[50] Hence, the sensitivity of a screening test should be defined with respect to all colorectal cancers existing within the population. Two methods are used for identifying prevalent cancers in a population. Total colonoscopy identifies prevalent cancer cases most efficiently, but is very difficult to perform on all asymptomatic persons and is not ethically acceptable.[27, 28] Another method is follow-up using cancer registries. Sensitivity is calculated differently according to the accuracy of identifying prevalent cancer cases of each method.[27, 50] Sensitivity calculated with the follow-up method using cancer registries is higher than that estimated using colonoscopy on all test-negative subjects.[27] Calculation using the follow-up method leads to overestimation of sensitivity due to its inability to accurately identify prevalent cancer cases, which is the most important factor for calculating sensitivity.[27, 50] On the other hand, sensitivity calculated by performing diagnostic investigation on all persons in the population should be underestimated due primarily to overdiagnosis of cancer, or a long latent phase in non-fatal cancers that allows long-term survival.[51] Sensitivity also varies among studies according to the definition of the stage of the target cancer and that of the duration of the preclinical stage.[27] Therefore, sensitivities estimated differently should be compared cautiously.[27]

Sensitivity based on colonoscopy on all subjects

Only a few studies of relatively small numbers of individuals have investigated the sensitivity of FOBT based on endoscopic findings in all subjects including test-negative subjects.

The conventional chemical test, the Hemoccult test, appears to have poor sensitivity based on colonoscopic findings for identifying prevalent cases. Ahlquist *et al.* reported that the Hemoccult test yielded only 26% positive test results in patients with colorectal cancer as identified by colonoscopy in an asymptomatic population at high risk.[52] Other reports have shown similar sensitivities of the Hemoccult test under similar conditions (27–47%,[13] 25%[53] and 23%[54]). Several investigators have concluded that FOBT is inadequate as a screening test on the basis of the sensitivity of Hemoccult and HemoQuant as shown by those studies.[52, 55]

Iwase conducted a study to compare RPHA test results with sigmoidoscopic findings.[13] Nearly 6,000 asymptomatic, average-risk persons who underwent multiphasic general check-ups including flexible sigmoidoscopy were tested for occult blood in stools by RPHA. Sensitivities for cancers distal to the sigmoid colon were calculated to be 67%, 78%, and 89% for 1-day, 2-day and 3-day IFOBT, respectively (Table II), whereas that of the Hemoccult test was only 33%.[13] A one-day testing of RPHA showed higher sensitivity than the standard 3-day Hemoccult test (Table II).[13] Another study based on colonoscopy in 808 asymptomatic subjects at high risk of colorectal cancer showed the test sensitivities of the 3-day RPHA test to be 70% and 44% for colorectal cancer and large (1 cm or greater diameter) adenomas, respectively. The corresponding values of the Hemoccult test,

Table II. Sensitivity and Specificity of FOBTs for Cancers within Reach of Flexible Sigmoidoscopy (FS) in 5,715 Asymptomatic Individuals Who Underwent FS as Well as FOBTs

	No. of subjects with cancer within reach of FS	No. of subjects with positive tests by FOBTs				
		RPHA			Hemoccult test	
		1-day[a]	2-day	3-day	3-day	3-day-m[b]
Invasive cancer[c]	9	6	7	8	3	6
Intramucosal cancer	6	3	5	5	1	1
All subjects	/	172	286	391	97	410
(n=5,715)		(3.0%)	(5.0%)	(6.8%)	(1.7%)	(7.2%)
Sensitivity[d]	/	66.7%	77.8%	88.9%	33.3%	66.7%
		(60)	(80)	(86.7)	(26.7)	(46.7)
Specificity	/	97.2%	95.2%	93.4%	98.4%	92.9%

RPHA: immunochemical hemagglutination test.

a) Results of specimens on first day of 3 days.

b) Results when trace reaction was judged as positive.

c) Nine patients consist of one each with Dukes B and C, and 7 with cancer limited to the submucosal layer.

d) Sensitivities for cancers including intramucosal cancer are given in parentheses. From Iwase.[13]

Table III. Sensitivity and Specificity of FOBTs Based on Follow-up Method in Identifying Colorectal Cancer Cases in an Asymptomatic Population

Reference	Screening test	No. of screenees	Se	Sp
Hiwatashi *et al.*[a)57]	one-day RPHA	40,267	71.4	97.7
	two-day RPHA	43,465	84.7	96.6
	three-day RPHA	15,767	90.9	96.9
Nakama and Kamijo[58]	one-day or two-day IFOBTs	3,365	90.9	95.6
Thomas *et al.*[59]	three-day Hemoccult	27,328	64.9	98.3
	six-day Hemoccult	10,890	73.5	98.2
Allison *et al.*[60]	three-day RPHA[b)]	7,493	68.8	94.4
	three-day HO[b)]	8,065	37.1	97.7
	three-day HO-Sensa[b)]	7,904	79.4	86.7

Abbreviations: Se, sensitivity for CRC; Sp, specificity; RPHA, immunochemical hemagglutination test; HO and HO-Sensa, "Hemoccult" and "Hemoccult-Sensa" (SmithKline Diagnostics, San Jose, CA).
a) Data from cooperative study of the research group organized by the Japanese Ministry of Health and Welfare.
b) The number of screenees who completed all three specimens was significantly lower for RPHA than for the Hemoccult test and Hemoccult-Sensa (5,044 vs. 7,409 and 7,575). Thus, sensitivity for RPHA is underestimated in comparison with the other two.

which was performed in half of the experimental population, were only 33% and 18%, respectively.[56] The Hemoccult sensitivities obtained in these two studies are consistent with those of previous reports,[52-54] clearly indicating that the sensitivity of the RPHA test is higher than that of the Hemoccult test.

Sensitivity based on the follow-up method

As an alternative to performing colonoscopy on all persons in the population, another method for identifying prevalent cases of colorectal cancer is to follow-up all test-negative subjects in order to determine how many cancers turn up amongst them,[27] using cancer registries. Sensitivities measured on the basis of follow-up of test-negative subjects are summarized in Table III. It was found that the sensitivities were approximately 70–90% for 1-day, 2-day, and 3-day IFOBT. These values are higher than those reported for the standard 3-day Hemoccult test.[57-60]

These studies indicate that the sensitivity of Hemoccult is insufficient for colorectal cancer, and that IFOBT is more sensitive than the Hemoccult test in known colorectal cancer subjects and in an asymptomatic population.

3. Population screening projects

Comparison of yields between IFOBT and the Hemoccult test

A population screening trial on a limited scale was first reported by Armitage using EIA, which detected a higher number of cancer subjects (positivity rate, 8%) than the Hemoccult test.[26] The present author conducted an initial screening program using the RPHA test developed and prepared in our laboratory, which identified two cancer cases in the 1% of subjects with positive test results among 1,712 average-risk persons.[11] The Hemoccult test, performed concomitantly without dietary restriction, exhibited a positivity rate of 7% but failed to identify any cancer subjects. Several other reports have compared the yields between IFOBT and the Hemoccult test by using the same specimens for FOBTs (Table IV).[13, 61-63] IFOBT showed a higher cancer detection rate than the Hemoccult test in actual population screening programs, although the positivity rates differ among the reports.[11, 13, 26, 61-67] The compliance rate was sufficiently high for IFOBT (Table IV).[11, 13, 65, 66] All of the studies were conducted under uncontrolled designs, and therefore could not be compared with each other and could not afford evidence of effectiveness. In order to investigate the feasibility of large-scale population screening using IFOBT, we conducted colorectal cancer screening using the RPHA test in conjunction with a gastric cancer mass survey program enrolling more than 30,000 subjects per year. Results similar to those in the smaller trials were obtained, with a compliance rate of approximately 80%.[11, 65] The results demonstrate the feasibility of performing a screening program on a large scale.

Specificity of IFOBT in a screening program

Attention should be drawn to the higher positivity rates obtained for IFOBT than for the Hemoccult test in several studies (Table IV).[26, 61, 62] The causes of these results have not been fully clarified. As mentioned above, factors that can produce false-positive results in IFOBTs include inadequate specificity of anti-Hb antibody and several technical factors.[29] This shortcoming is attributable to an excess amount of specimen. The positivity rate in specimens provided by screenees was 2 to 3 times

Table IV. Population Screening Results with Both IFOBT and Hemoccult Test, or IFOBT Alone

Reference	IFOBT[a]	No. of screenees	Compliance (%)	Positivity rate[b] (%)	No. of cancer detected[b]
Armitage *et al.*[26]	FECA EIA-3	1,304	44	8.1 (3.1)	5 (3)
Saito and Yoshida[11]	RPHA-1	1,712	90	1.1 (7.9)	2 (0)
Iwase[13]	RPHA-1	5,715	89	3.0 (1.7)	11 (5)
Frommer *et al.*[61]	SRID-6	1,328	/	9.1 (4.4)	19 (11)
Robinson *et al.*[62]	RPHA-3	1,489	37	9.7 (1.1)	9 (1)
Petrelli *et al.*[63]	RPHA-3	8,933	23	4.9 (5.1)	21 (16)
Häkkinen *et al.*[64]	EIA-3	6,878	66	7.7	7
Kawaguchi *et al.*[d][65]	RPHA-1	92,159	83	2.2	59[c]
	RPHA-2	40,149	78	3.0	38
Fujita *et al.*[66]	RPHA-3	15,488	84	3.2	30[c]
Hiwatashi *et al.*[67]	RPHA-1	5,338	48	2.5	27[c]
	RPHA-2	2,633	53	3.2	14

Abbreviations: IFOBT, immunochemical fecal occult blood test; EIA, enzyme-linked immunosorbent assay; SRID, single radial immunodiffusion.
a) Number of specimens tested.
b) Values in parentheses are for Hemoccult test.
c) Including subjects with intramucosal cancer.
d) Results from total of 4 consecutive annual screenings in the population.

higher than that of specimens smeared in the laboratory for RPHA testing (2–3% vs. 1%).[11, 65] Indeed, the positivity rate decreases after specimen-smeared cards with large stool samples prepared by screenees were re-prepared properly in the laboratory. The need for a better tool enabling preparation of a constant amount of stool must be stressed. Another possible cause of false-positive results relates to the readability of the test results.[32] Skilled technicians obtain lower and more stable positivity rates than unskilled persons. Readability is important, especially in a laboratory without a technician familiar with the test.[32] Some investigators have noted that the positivity rate in a population screening program could be decreased from 8.2% to 3.1% by excluding (±) results from positive results, without affecting the cancer detection rate,[68] reflecting the difficulty of visual interpretation of test end-points.[32] A test device such as an autoreader, which enables high reproducibility for some test kits, may be necessary.

Results of a nationwide program

In 1992, a screening program using IFOBT was adopted as a public health policy in Japan.[11, 14] Persons 40 years of age or older are recommended to participate in the screening program using 2-day IFOBT, which was selected based on its well-balanced sensitivity and specificity according to early studies.[49] Fees for screening are partially met by the government, and those for diagnostic investigation are paid by the subjects using national health insurance. More than 2.5 million individuals participated in the screening program in 1992.[11, 69] Data obtained from 1.08 million persons are summarized in Table V. Of the 76,000 individuals (7.0%) that showed positive test results, 70% underwent diagnostic investigation. Colorectal cancers were found in 2,000 of the subjects (0.2%), and 51% of the cancers were limited to the mucosa or carcinoma *in situ* ('m' cancers) (Table V). Excluding these 'm' cancers, 990 cancers were detected, of which approximately 50% were limited to the submucosal or muscular layer without lymph-node or distant metastasis. Of the cancer subjects, 55% were treated by surgery and 45% by endoscopic polypectomy.[69]

Table V. Population Screening Yields by the Screening Program Covered by the Law for the Aged in 1992, Using Immunochemical Tests

No. of participants	1,083,097
No. of subjects with positive tests (%)	76,323 (7.0%)
No. of subjects who underwent work-up	53,536 (70.1%)
No. of subjects with colorectal cancer	2,017 (0.2%)
No. of early cancers[a]	1,139

a) Cancers limited to the mucosal layer or the submucosal layer. From Saito and Yoshida[11] with permission.

Evaluation of screening programs for colorectal cancer using IFOBTs

The colorectal cancer screening program in Japan has been shown to be feasible as a nationwide program to reduce mortality. However, cancer screening programs can harm a population.[27] Thus, each cancer screening

program must be evaluated in order to determine whether it is effective before it is implemented as a national public health policy. Four parameters related to the effectiveness of screening need to be examined,[1, 28] including cancer staging, survival, mortality, and cost effectiveness.[1] Mortality is the most important of these factors.

1. Biases relevant to evaluation of screening

Cancers detected by screening tend to be in earlier stages than those routinely diagnosed.[50] This tendency was apparently confirmed in colorectal cancer screening using FOBT.[28, 70–73] However, early detection does not necessarily indicate the effectiveness of the screening program, due to several major biases.[27, 28]

Although detection of earlier stage cancers with improved survival is a critical requirement for an effective screening program, early detection can be achieved without an accompanying increase in survival,[1, 27] because screening may merely move the time of diagnosis forward, without moving the time of death backward (lead time bias).[27] A slow-growing tumor with a favorable prognosis is more likely to be detected by screening.[27] Accordingly, screen-detected cases will have a seemingly superior rate of survival (length bias).

In addition, the underlying risk of developing a disease or a cancer might differ between responders and non-responders to screening.[27, 50] Persons who elect to participate in the screening may be health-conscious, and thus may be at lower risk for developing the disease.[27, 50] Under such conditions, cancer is more likely to be present in the non-responder groups. Hence, the responder group has a better survival rate than the non-responder group (self-selection bias).

2. Mortality reduction

For the above reasons, evaluation of the effectiveness of a screening program should be performed with mortality as the outcome parameter.[1, 50] A randomized controlled trial (RCT) is the best method to evaluate the screening program.[1, 27] While various such trials are being conducted,[70–72] a study from Minnesota showed that annual FOB screening by Hemoccult resulted in a 33% decrease in the 13-year cumulative mortality rate from colorectal cancer, compared to a control group (Table VI).[6] The effectiveness of a screening program using an FOBT is demonstrated by this study. A case-control study suggested similar effectiveness.[74] However, the magnitude of the program effectiveness demonstrated by the RCT appears to be rather small from the viewpoint of cost-effectiveness.[7, 8] Other ongoing RCTs using the Hemoccult test may be completed within a few years, but a mortality reduction significantly greater than that of the Minessota trial is unlikely to be observed. Meta-analysis of the mortality data from the RCTs suggested only a 19% reduction in mortality.[75]

There is indirect but relatively strong evidence indicating that screening by sigmoidoscopy would reduce the mortality.[76, 77] Furthermore, it has been suggested that the effect might continue for 10 years after screening.[76] Unfortunately, this approach is not feasible due to poor acceptability of screening sigmoidoscopy.[78] An IFOBT possessing better performance characteristics is likely to achieve higher relative cost-effectiveness. IFOBT, and especially the RPHA test, is a promising candidate.[11, 32]

Because screening with an IFOBT is widespread due to its adoption as a public health policy, conducting an RCT in Japan would be very difficult.[11, 14, 15] An alternative way to demonstrate the efficacy of a screening program is to perform a case-control study in an area where screening is widely performed.[79] Case-control studies provide information on the magnitude of the benefit of screening, especially in relation to the frequency of screening.[80] Such information is not always obtained from an RCT.[80] A cooperative case-control study in areas where colorectal cancer screening had been performed with the Hemoccult test during the first half of a 10-year period and subsequently with IFOBTs in the second half of the

Table VI. Magnitude of Mortality Reduction by FOBT Screening

Reference	Year	Screening test	Study design	Mortality rate reduction (%)
Selby *et al.*[74]	1992	Hemoccult[a]	Case-control	30
Mandel *et al.*[16]	1993	Rehydrated Hemoccult[a]	RCT	33
Hiwatashi *et al.*[82]	1993	IFOBTs and Hemoccult[b, c]	Case-control	76
Wahrendorf *et al.*[5]	1993	Hemoccult[a]	Case-control	57[d]
Saito *et al.*[15]	1995	RPHA[c]	Case-control	60

a) Three-day testing.
b) Partially by any of 4 immunochemical tests and partially by Hemoccult test.
c) One-day testing.
d) Females only.
Rehydrated Hemoccult test: Hemoccult testing after hydration with one drop of water. Sensitivity is improved by hydration, but specificity is worsened.

period was conducted on 57 deceased cases of colorectal cancer and 171 controls.[81] This study suggested that the risk of dying of colorectal cancer was reduced by 70% by annual screening. One part of this cooperative study was reported by Hiwatashi *et al.*, with a mortality rate reduction of 76%.[82] However, the main contributor to the efficacy could not be determined in the study, because more than two kinds of FOBTs were used.

In 1993, a case-control study was conducted in order to evaluate the effectiveness of screening programs that use IFOBT alone.[15] This study was performed in areas where annual screening had been performed using only the 1-day RPHA test without a previous colorectal cancer screening program, under conditions intended to preclude confounding biases.[15] Analysis was performed using conditional logistic regression models. Results of the study for 193 deceased cases of colorectal cancer and 577 controls, matched by age, sex and residential area, indicated an approximately 60% reduction in mortality rate due to screening with the 1-day RPHA test (Table VI).[15] The mortality rate reduction was calculated to be a maximum of 84% when possible symptom-derived subjects were excluded; inclusion of such symptom-derived subjects is known to lead to underestimation of effectiveness.[83] The results also suggested that the risk remains low for 3 years after the final screening test.[15] A potential major confounding bias that might have influenced the results of this study was the self-selection bias, i.e., a lower incidence of colorectal cancer in responders than in non-responders to screening might have exaggerated the effectiveness. However, the effectiveness is not mainly attributable to this bias, because the colorectal cancer incidence was estimated to be higher in responders than in non-responders even after excluding all screen-detected cases (unpublished results). This evidence might validate the effectiveness suggested by the case-control study.[84]

The magnitude of the mortality rate reduction is also supported by a study comparing survival between subjects with screen-detected colorectal cancer and those with clinically diagnosed colorectal cancer using Cox's proportional hazards model. After adjusting for factors relevant to prognosis, it was suggested that the mortality rate would be reduced by 60% by screening using 1-day RPHA testing.[85] A time-trend study that compared the standardized mortality ratio in a study area to that in a control area before and after the screening program showed a significant decrease in the mortality ratio in the study area after the program was adopted.[86]

Evidence obtained from the case-control study described above met the criteria for Quality of Evidence of II-2 by the U.S. Preventive Services Task Force,[87] corresponding to category B in the Strength of Recommendation, which states that "There is fair evidence to support the recommendation of screening."

3. Cost-effectiveness

Even if the screening program is highly effective and exhibits a strong impact on mortality, screening cannot be accomplished when a society is not willing to pay the cost of the program. Cost issues involve many complex aspects, such as hidden costs, which include the costs assigned to the patients' time and inconvenience, as described by Simon.[28] Moreover, cost-effectiveness is dependent on disease prevalence, which varies among countries, and the costs of diagnosis and treatment, which also differ. Thus, obtaining evidence on cost-effectiveness that can be internationally generalized is difficult. However, we must consider whether the program is feasible and effective in terms of cost.

The average cost for the detection of colorectal cancer using IFOBT in the form of the RPHA test is almost one-third lower than that using the Hemoccult test, due to the higher detection rate of colorectal cancer with a lower positivity rate for the RPHA test than for Hemoccult.[11] The average cost for colorectal cancer screening is lower than that for the gastric cancer mass survey, which is the most widely accepted screening program in Japan (unpublished results). Two other studies have also shown that screening using IFOBT is far more cost-effective than that by the Hemoccult test. Shimbo *et al.* reported that a screening strategy using IFOBT had a cost-effectiveness ratio of 13,100 US dollars per year of life saved as compared to 28,500 US dollars for the Hemoccult test.[88] Tsuji *et al.* also reported that IFOBT is the most cost-effective option.[89] Thus, the screening program by IFOBT is considered valid in terms of cost-effectiveness in Japan, although more studies are needed to confirm this.

Conclusions

Screening for colorectal cancer by conventional FOBT has been shown to be effective for reducing mortality. However, the magnitude of its effectiveness is small in view of its low cost-effectiveness. IFOBT represents a promising screening test that might achieve greater effectiveness. Studies of sensitivity and specificity, including an ROC analysis, demonstrated the validity of IFOBT. The feasibility of the screening strategy with IFOBT has been demonstrated on the basis of better feasibility indicators of IFOBT than of conventional FOBT in population screening programs, i.e., IFOBT has higher sensitivity, specificity, and a similar or superior compliance rate than the Hemoccult test. The results of a nationwide screening program performed in Japan supported the feasibility and efficacy of this strategy for a large population. To achieve greater specificity, a sampling device that can provide a constant amount of specimen must be developed. The effectiveness of this approach is strongly

supported by case-control studies, which indicate a mortality rate reduction of 60% or more. Although potential biases inherent in observational studies may be partially responsible for the estimated effectiveness, the evidence obtained satisfies the II-2 criteria by the U.S. Preventive Services Task Force. Several well-designed observational studies are ongoing to confirm this effectiveness. In order to establish a screening method that has a strong impact on mortality and morbidity from colorectal cancer, issues such as the optimal screening interval should be further investigated.

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