Comparison of fecal occult blood assay by four companies

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Abstract: The principles of immunological fecal occult blood measurement are applied in methods such as reversed passive hemagglutination, e latex agglutination reaction, colloidal gold colorimetry, and immunochromatography. At present, a number of reagent kits that use these principles are available on the market. This study compares the clinical sensitivity and specificity of the reagents for the kits manufactured by four companies: Fujirebio Inc., Wako Pure Chemical Industries, Ltd., Azwell Co., Ltd. and Kyowa Medex Co., Ltd. Based on the sensitivity and specificity data thus established, we have also prepared an ROC curve for each reagent. On the basis of these results, we have determined the antibody specificity and cut-off values for the factors accounting for the disparity of the individual reagent kits and focused investigations on the fecal sampler.

When using the cutoff value for the clinical sensitivity and specificity recommended with each reagent kit, we did not find any major differences for the different assay methods. The sensitivities of the different methods were compared on the broad distinction between early and progressive cancer. The result for early cancer was 17 - 50% and for progressive cancer 83 - 92%. Thus there was a marked difference between the two. Sensitivity comparison made by dividing the subjects into the colon cancer and rectum cancer groups showed that while sensitivity in case of rectum cancer was slightly greater than that in case of colon cancer, there was no significant difference in evidence between the two groups. Specificity comparison revealed that the kits of manufacturers using a high fecal concentration in the fecal sampler after sampling (i.e., final fecal concentration) tended to have a lower specificity. The important factors that impact on the sensitivity and specificity of the various kits are the determination of the cut-off value, the accuracy of fecal sampling and the characteristics of the antibody that is used.

Keywords: Sensitivity; Specificity; Cut-off value; ROC analysis; quantification of Hb.

Introduction

The main assay methods of immunological fecal occult blood test are the reverse passive hemagglutination, the latex agglutination reaction, the colloidal gold colorimetry, and the immunochromatography, and a number of reagents based on these assay methods are available on the market. However, the standard values (cutoff values) of these reagents are determined specifically for each reagent and the fact is that the standard values differ from one reagent to another even though they may use the same measurement principle.[1,2)]

For this study, we used the fecal samples provided by colorectal cancer patients hospitalized in our clinic and by healthy control subjects. For the different assay kits, we compared their clinical sensitivity and specificity and concurrently we also compared the sensitivity and specificity as for each cancer location and depth. Based on the results, ROC curves were prepared to investigate whether the cutoff values had been appropriate. In addition, we considered the relevance of quantitatively assaying the fecal hemoglobin (Hb). The results are presented in this report.

Table 1 Cancer location and deepness based on endoscopic examination

Depth	Anus	Rectum	Colon	Cecum	Total
m	0	2	0	0	2
sm	0	2	2	1	5
mp	0	11	8	0	19
ss(a_1)	0	26	26	0	52
se(a_2)	0	5	0	0	5
si(ai)	0	1	0	0	2
Total	1	47	36	1	85

Table 2 Measurement principles for each reagent kit and Sensitivity in comparison

		Hem	JIA	NS	HM
Cutoff value	ng/mL	20	50	40	12
	μg/g of fecal sample		12.5	40	30
Detection incidence (No. of persons)		69	75	54	74
Sensitivity (%)		81.2	89.3	73.0	87.1

I. Methods

1. Subjects

The subjects for this study consisted of a group of 85 patients with colorectal cancer who visited this clinic for about 2 years from February 2000 to December 2001 and had given their consent to participate in this study, and of a group of 125 healthy controls who visited this clinic from November 2001 to January 2002. The colorectal cancer group of 85 patients had been diagnosed as having histologically proven cancer by endoscopy (44 males and 41 females). The normal control group consisted of 125 subjects (80 males and 45 females) diagnosed by endoscopy as being free of cancer but with adenoma, and 67 subjects (38 males and 29 females) had been diagnosed as being free of cancer and adenoma. ROC curves were prepared for 125 subjects found to have adenoma.

2. Apparatus and Reagents

Used as the reagent based on the reversed passive hemagglutination as the principle of measurement was the reagent IMMUDIA Hem-Sp (Fujirebio Inc., referred to as “Hem” below). The apparatus used was Fastic 401 and Fastic 404. Used as the reagent based on the colloidal gold colorimetry as the principle of measurement was Immuno-Gold Hem (Wako Pure Chemical Industries, Ltd., referred to as JIA below), and the Hemo Plate Auto II, Azwell Co., Ltd., “NS” below). JIA-HB2010 was used as the assay apparatus for JIA and NS-1000 for NS. Used as the reagent based on the latex agglutination reaction was HEMO AUTO (Kyowa Medex Co., Ltd., “HM” below) and HM-JACK

was used as the assay apparatus. All reagents and assay units were used in accordance with the instruction leaflets attached to them and the preset cutoff value was employed.

3. Fecal sampling

Both the colorectal cancer patients and the healthy control submitted stool specimens in the size of a finger tip at the laboratory of this clinic, and these specimens were used as samples. The laboratory technician collected the samples on the special samplers of the respective reagent kits. In the case of the Hem kit, fecal samples were taken in the special samplers prescribed for this reagent kit and assayed by the semiquantitative method using successive dilutions[3)4)]. In the case of JIA, the submitted specimens were stored cryogenically at -40°C. The fecal samples were sent to the manufacturer at a later date for analysis[5)-7)]. In the case of HM and NS, the submitted fecal samples were kept frozen at -20°C and later used at this clinic for sampling in the special samplers for the respective reagent kits and measurement at our clinic[8) 9)].

II. Results

1. Endoscopic findings of colorectal cancer patients

Table 1 shows the cancer locations and depth data obtained from the endocscopic examinations of the colorectal cancer patients.

The group included 47 patients, 55% of the total, whose cancer was located in the rectum and 36 patients or 42% with colon cancer. The total of patients with cancer in the colon and rectum thus amounted to approximately 97% of all patients. By depth of cancer, 52 patients or approximately 61% were ss (a_1) 19 or approximately 22% mp, and 5 or approximately 6% each sm and se (a_2), respectively, while 2 patients or approximately 2% each were m and si (ai), respectively.

2. Comparison of sensitivity by reagents

Comparison of sensitivity by the different assay methods revealed no substantial differences. Moreover, sensitivity comparison for all of the reagent kits showed that Hem was 81.2%, JIA 89.3%, NS 73.0%, and HM 87.1% (Table 2). Although JIA and NS use colloidal gold colorimetry as the measurement principle it was possible to discover a substantial difference in sensitivity between them.

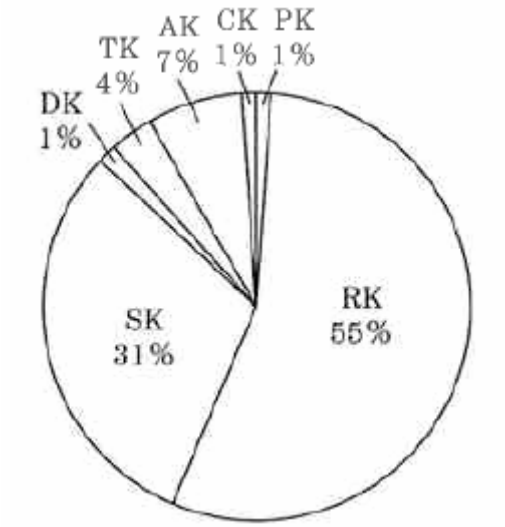

Fig. 1 Patient breakdown by cancer location

3. Sensitivity comparison by cancer location

The patient breakdown by cancer location shows that in 1 patient, the cancer was situated in the anal canal (PK below), in 47 patients in the rectum (RK below), in 26 patients in the sigmoid (SK below), in 1 patient in the descending colon (DK below), in 3 patients in the transverse colon (TK below), in 6 patients in the ascending colon (AK below) and in 1 patient in the cecum (CK below). Thus the total of RK plus SK accounts for approximately 86% of the entire colorectal cancer group, with the cases of rectal and sigmoid cancer representing the majority of cancers detected in this study. (Fig 1)

Table 3 Sensitivity comparison by cancer location

Cancer location	Patients (Persons)	Hem		JIA		NS		HM	
		Number detected (Persons)	Sensitivity (%)	Number detected (Persons)	Sensitivity (%)	Number detected (Persons)	Sensitivity (%)	Number detected (Persons)	Sensitivity (%)
PK	1	1	100.0	1	100.0	1	100.0	1	100.0
RK	47	38	80.9	41	89.1	30	85.0	40	85.1
SK	26	21	80.8	24	92.3	18	78.3	23	88.5
DK	1	1	100.0	1	100.0	1	100.0	1	100.0
TK	3	2	66.7	2	66.7	2	66.7	3	100.0
AK	6	5	83.3	5	83.3	2	40.0	5	83.3
CK	1	1	100.0	1	100.0	0	0.0	1	100.0
Total	85	69	81.2	75	89.3	54	73.0	74	87.1

Measurements were carried out on this total of 85 colorectal cancer patients to compare the sensitivity of each reagent kit as a function of the cancer location. While the sensitivity for PK and DK was low at only 1 patient each it was 100.0% for all of the four manufacturers. Sensitivity for RK was 80.9% for Hem, 89.1% for JIA, 85.0% for NS, and 85.1% for HM. Thus, JIA had the highest sensitivity. Sensitivity for SK was 80.8% for Hem, 92.3% for JIA, 78.3% for NS, and 88.5% for HM. Thus, JIA had the highest sensitivity. Sensitivity to TK was 66.7% for Hem and JIA and NS, 66.7% for NS, and 100.0% for HM. Thus, HM had the highest sensitivity. Sensitivity to AK

was 83.3% for Hem, JIA and HM, and 40.0% for NS. Sensitivity to CK was 0.0% for NS and 100.0% for Hem, JIA and HM, given the fact that the patient number at 1 person only was too small (Table 3).

4. Sensitivity Comparison by Cancer Depth

The patient number by cancer depth was 2 for m cancer, 5 for sm cancer, 19 for mp cancer, 52 for ss (a_1) cancer, 5 for se (a_2) cancer, and 1 for si (ai) cancer (Table 4). The total of mp cancer plus ss (a1) cancer thus accounted for a majority of 84% of all patients (Fig. 2).

For this group of 85 colorectal cancer patients, measurements were made to compare the sensitivities of the different reagent kits by depth of the cancer. The sensitivity for m cancer was low at only 2 patients. It was 50.0% for Hem, 0.0% for JIA, 0.0% for NS and 50.0% for HM. Sensitivity to sm cancer was 40.0% for Hem, 20.0% for NS, and 60% for both JIA and HM. Sensitivity to mp cancer was 73.7% for Hem, 84.2% for JIA, 62.5% for NS, and 89.5% for HM, with HM having the highest sensitivity. Sensitivity to ss (a_1) cancer was 88.5% for Hem, 96.2% for JIA, 84.8% for NS and 90.4% for HM, with JIA having the highest sensitivity. Sensitivity to se (a_2) cancer was 80.0% for Hem, JIA, and HM and 50.0% for NS. Sensitivity to si (ai) cancer was 100.0% for the kits of all four manufacturers, although it should be noticed that the number of patients was too small at only 2 (Table 4).

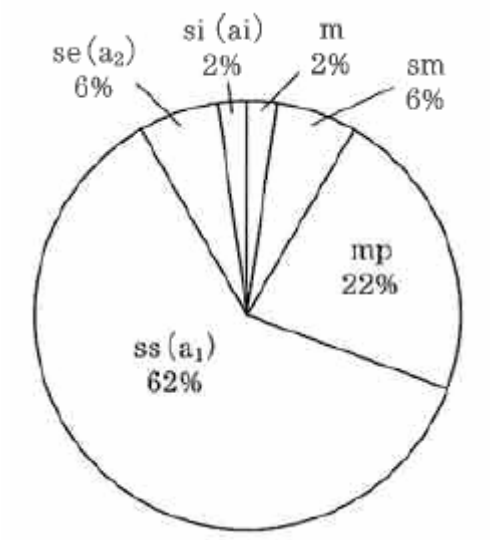

Fig. 2 Patient breakdown by cancer depth

Table 4 Sensitivity comparison by cancer depth

Depth	Patients (Persons)	Hem		JIA		NS		HM	
		Number detected (Persons)	Sensitivity (%)	Number detected (Persons)	Sensitivity (%)	Number detected (Persons)	Sensitivity (%)	Number detected (Persons)	Sensitivity (%)
m	2	1	50.0	0	0.0	0	0.0	1	50.0
sm	5	2	40.0	3	60.0	1	20.0	3	60.0
mp	19	14	73.7	16	84.2	10	62.5	17	89.5
ss(a_1)	52	46	88.5	50	96.2	39	84.8	47	90.4
se(a_2)	5	4	80.0	4	80.0	2	50.0	4	80.0
si(ai)	2	2	100.0	2	100.0	2	100.0	2	100.0

Total	85	69	81.2	75	89.3	54	73.0	74	87.1

Table 5 Means of Hb fecal concentration measured with each reagent kit by cancer depth.

Depth	Patient number (Persons)	Hem	JIA	NS	HM
m	2	0.0	1.3	0.0	166.1
sm	5	52.0	179.7	70.4	234.2
mp	19	96.8	335.3	416.4	720.6
ss(a_1)	52	125.8	2,257.4	2,682.8	786.8
se(a_2)	5	104.0	517.2	109.0	435.6
si(ai)	2	160.0	315.5	746.5	1,551.1

(Unit:μg/g of stool)

5. Quantification (numerical value) of Hb

Table 5 shows the mean values of Hb in feces determined with the different kits by cancer depth. Although in case of ss (a_2) and si (ai) it was not possible to obtain satisfactory results because of the small patient number it was found that the average values tended to increase as the cancer's progression to the greater depth. In case of ss (a_1), JIA and NS which are based on the colloidal gold colorimetry showed elevated values. Comparison of the cancer depth and the mean values for the Hb concentration shows that NS and JIA showed maximum values for ss (a_1) and that the mean value for si (ai) are lower than those for ss (a_1). HM tended to produce higher mean measurement values with progressing depth of the cancer. In contrast, the Hem kit, which is of a reverse passive hemagglutination, showed low values for all cancer depth. (Table 5)

Table 6 Specificity Comparison by manufacturer

Endoscopic findings	Number of patients (Persons)	Hem		JIA		NS		HM	
		Negative incidence (Persons)	Specificity (%)	Negative incidence (Persons)	Specificity (%)	Negative incidence (Persons)	Specificity (%)	Negative incidence (Persons)	Specificity (%)
No findings	67	65	97.0	61	91.0	66	98.5	66	98.5
Incl. adenoma	125	120	96.0	113	90.4	122	97.6	121	96.8

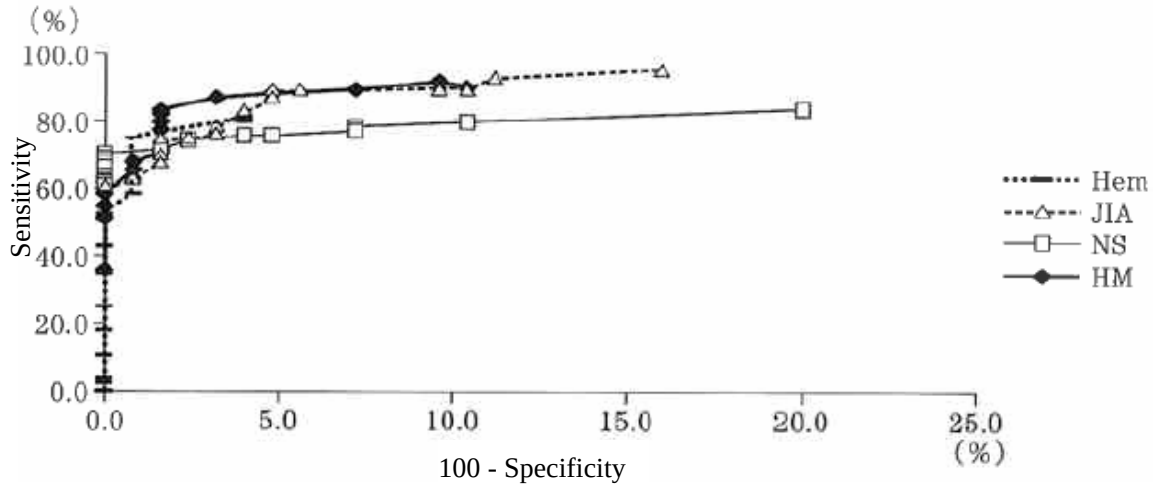

Fig. 3 ROC curve by manufacturer

Table 7 Comparison of Sensitivity and Specificity at the recommended cutoff values and at the hypothetical cutoff value.

	JIA			NS			HM		
	Cutoff values (ng/mL)	Sensitivity (%)	Specificity (%)	Cutoff values (ng/mL)	Sensitivity (%)	Specificity (%)	Cutoff values (ng/mL)	Sensitivity (%)	Specificity (%)
Recommended cutoff value	50	89.3	90.4	40	73.0	97.6	12	87.1	96.8
Hypothetical cutoff value	60	89.3	95.2	20	78.4	92.8	10	88.1	95.2

6. Comparison of Specificity

The specificity of the reagent kits was investigated for the 67 cases without endoscopic findings. Comparison of the specificity values for the different reagent kits shows that Hem has a specificity of 97.0%, JIA of 91.0%, NS of 98.5% and HM of 98.5%, indicating that Hem, NS and HM all have a high specificity of 97% or more. Even with an extended definition of healthy, normal control group to “include adenoma”, the specificity data for Hem is 96.0%, for JIA 90.4%, for NS 97.6% and for HM 96.8%, with NS having the highest specificity. (Table 6)

7. ROC Analysis

i) ROC Curve

ROC curves were established for each of the kits. Based on the results of the present study, we investigated the optimum cutoff value for each kit (Fig. 3). The sensitivity and 100-specificity (%) at the cutoff values recommended by the manufacturers are: For Hem 81.2% and 1.4%, for JIS 89.3% and 9.6%, for NS 73.0% and 2.4%, and for HM 87.1% and 3.2%, respectively. On an overall assessment including both the sensitivity and specificity the HM reagent kit was the most favorable. However, when using the minimum value of $[(1 - \text{sensitivity})^2 + (1\text{-specificity})^2]$ as the hypothetical cutoff value it was found that the cutoff value that is different from the cutoff value currently recommended by the reagent kit manufacturers tends to improve both the sensitivity and the specificity (Table 7)

In case of HM, no major difference was found between the present cutoff value and the hypothetical cutoff value. In contrast, for NS which had a low sensitivity it was possible to detect a large difference between the present cutoff value and the hypothetical cutoff value. Yet while the sensitivity did increased at the hypothetical cutoff value, the specificity was found to decrease. In case of JIA which had a low specificity it was possible to discover that while there was no major difference between the cutoff values, specificity tended to improve at the hypothetical cutoff.

Comparison of the correct diagnosis rates at the recommended cutoff value and at the hypothetical cutoff value has shown that while changing the cutoff value led to an improvement in the correct diagnosis rate in the case of JIA, no significant change in the correct diagnosis rate was found for NS and HM (Table 8).

Table 8　Comparison of correct diagnosis rates at recommended cutoff value and the hypothetical cutoff value

	JIA	NS	HM
Correct diagnosis rate at recommended cutoff value (%)	90.0	88.4	92.9
Correct diagnosis rate at hypothetical cutoff value (%)	92.8	87.9	92.4

2) Results by cancer location

We have plotted the sensitivity and 100 - Specificity for rectal cancer and colon cancer using the cutoff values recommended by the different manufacturers for their respective reagent kits. The plots obtained with the different reagent kits for rectal cancer and colon cancer do not digress from each other and show almost an identical performance. The kits of the four manufacturers also have in common that their sensitivity for colon cancer is greater than that for rectal cancer to some minor extent of a few percent (Fig. 4).

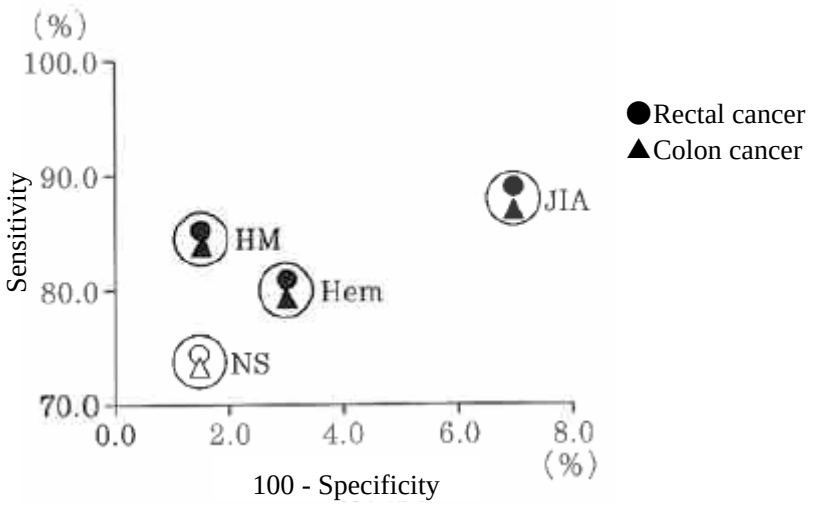

Fig. 4　Sensitivity and Specificity by cancer location

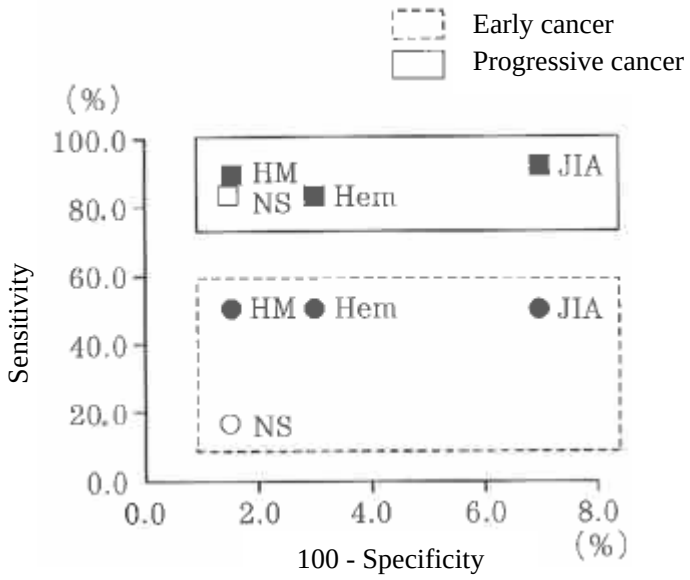

Fig. 5 Sensitivity and Specificity by cancer depth

3) Results by cancer depth

For all reagent kits, the sensitivity and 100 - specificity were plotted only with regard to an early cancer (m and sm cancer) and a progressive cancer group. Although the results showed no difference in specificity for the early and advanced cancer groups the sensitivity results were different for each group. In the advanced cancer group, all of the four reagent kits had a sensitivity in excess of 80% while their sensitivity towards early cancer was only 50% or less, with NS having the lowest sensitivity at only 17% (Fig. 5)

III. Discussion

This study was conducted to investigate the sensitivity and specificity of immunological assay of occult blood in the feces using the reagent kits of four manufacturers. The cancer locations of the 85 subjects with colorectal cancer broke down into 55.3% rectal cancer and 30.6% sigmoid colon cancer. These two cancer groups accounted for roughly 86% of the total patient number. It is wellknown that the preferential sites of onset of colorectal cancer are the rectal and the sigmoid colon, and the present study has confirmed this tendency[9) - 11)]. A certain, though only minor discrepancy was discovered in this study among the different reagent kits. Table 9 and Fig. 6 show the correlation that exists between the fecal concentration in the sampler after sampling (final fecal concentration) calculated from the fecal sample weight in the samplers of the respective reagent kits and the weight of the buffer solution versus the sensitivity and specificity.

Table 9　Sample weight in fecal sampler of the reagent kits and weight of stool dissolving solution

	Hem	JIA	NS	HM
Fecal sample weight (mg)	0.2	4	3	0.5
Stool dissolving solution volume (mL)	1	1	3	1.25
Final fecal concentration (mg/mL)	0.2	4	1	0.4

Table 10　Final fecal concentration in samplers of each reagent kit and fecal weight carried over to the reaction system.

	JIA	NS	HM
Final fecal concentration (mg/mL)	4	1	0.4
Sample amount (μL)	24	25	12
Fecal amount carried over into the reaction system (μg)	96	25	4.8
Final reagent volume (μL)	336	175	288
Fecal concentration carried over into the reaction system (mg/mL)	0.29	0.14	0.02

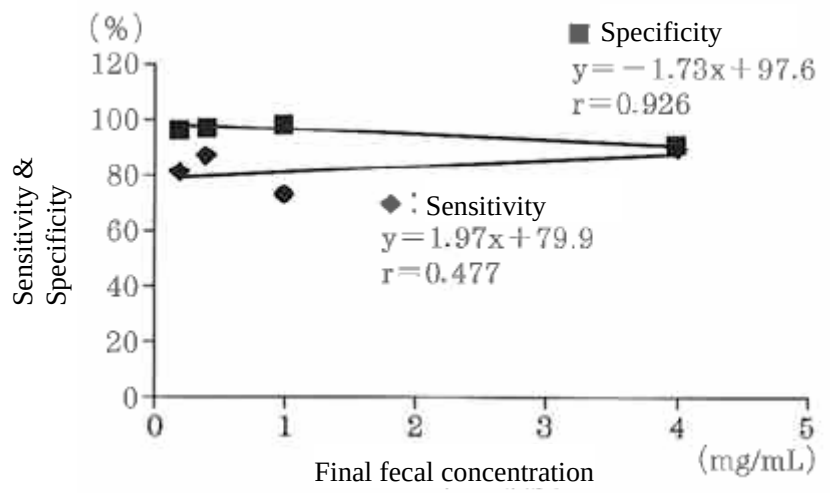

Fig. 6　Final fecal quantity versus sensitivity and specificity for each reagent kit.

From Fig. 6 it can be seen that although there is no correlation between the final fecal amount and the sensitivity there is a strong correlation in terms of the final fecal amount and the specificity at r = 0.926 although the value n is small. The greater the final fecal concentration after sampling the more the Hb amount taken into the sampler will also increase and so will the residues in the feces. Table 10 shows the fecal concentration in the reaction system calculated from the final fecal concentration, the sample amounts of the respective reagent kits and the reagent quantities.

While, in the case of reagent kits JIA and HM, it was not possible to detect a substantial difference in the sample quantity and reagent quantity ratio there was a significant difference in specificity between them. The final fecal concentration in the sampler for JIA and HM differed by a factor of 10 and similarly the fecal concentration in the reaction system also differed by a factor of 10 or more. This was considered due to the effect of the final fecal concentration in the sampler and the fecal concentration in the reaction system. Even on a comparison of the final fecal concentration and the fecal concentration in the reaction system in the case of NS and JIA it was found that NS had a

lower final fecal concentration in the sampler and a lower fecal concentration in the reaction system but a higher specificity. Comparison between NS and HM, however, showed that while NS has a higher final fecal concentration and also a higher fecal concentration in the reaction system it also had a higher specificity albeit by only a small margin. These results suggest that there may be another factor that has an impact on the reagent's specificity than the final fecal concentration in the sampler.

No correlation was found to exist between the sensitivity and the final fecal concentration in the respective samplers. In the case of NS, the final fecal concentration was 1mg/mL. Yet although its final fecal concentration in the samplers was 2.5 times greater than in the case of HM, HM did exhibit a higher sensitivity. Furthermore, comparison between HM and JIA revealed that although HM had a final fecal concentration in the fecal sampler only 1/10 that of JIA, there was no substantial difference in sensitivity between these two reagent kits. These findings indicate that in contrast to the specificity, the final fecal concentrations for each reagent kits has little influence on sensitivity. The reagent kits used for the present study are all based on agglutination as the measurement principle. Sensitivity may also be affected by the size of the carrier particles used by the respective reagent kits. Similarly, the specificity and titer of the antibody binding to the carrier particles may likely have an effect on sensitivity[10].

In this study, we have examined the sensitivity and 100 - specificity only by dividing the subjects into an early cancer group and an advanced cancer group. The results have shown that the early cancer group had a lower sensitivity than the advanced cancer group. It has been reported earlier that as the cancer progresses so the Hb concentration in the stool will increase[12) - 14)]. This findings was corroborated in this study which showed that the fecal Hb concentration tended to increase with cancer progression, as shown in Table 5. The particular feature of early cancer is that the intermittent hemorrhaging takes place from the lesion in minor quantities. This characteristic feature of early cancer is a major influencing factor with regard to the sensitivity by cancer depth. Sensitivity in the detection of early cancer using examination of occult blood in the feces may conceivably be improved by fixing the cutoff value at a lower value. Yet this will lead to a decrease in specificity[15]. After allowing for the fact that intermittent hemorrhaging from the lesions takes place in early cancer, it does seem necessary for the detection of early cancer and the proper assessment of the condition not only to conduct immunological occult blood examinations in the feces but also to give full consideration to other examination data.

Comparison of the sensitivity of the various reagent kits for early and advanced cancer has demonstrated that there is no major differences among the reagent kits concerned in advanced cancer and that NS has a lower sensitivity than the other reagent kits for early cancer. Although not shown in the data, it can be seen that the one of the four cases of sm not recognized by the NS kit has a concentration of 25ng/mL, and it could have been picked up as the hypothetical cutoff value of 20ng/mL based on the results of this study. This suggests that one of the factors as to why NS has a lower sensitivity for early cancer may be due to the effect of the cutoff value setting.

Comparison of the sensitivity results for colon cancer and rectal cancer indicated that there were no significant differences among any of the reagent kits. As stated earlier, however, the majority of the cancers investigated in this study were located in the rectum and colon. Further, comparison in terms of the cancer depth detected showed that almost all of them were of the advanced cancer. It has already been pointed out that the fecal Hb concentration will increase with cancer progression. The reason why no major difference was detected between colon and rectal cancer in the sensitivity comparison by cancer location my be attributed to the fact that almost all of the cancers discovered in this study were advanced ones occurring in the colon and rectum. This suggests that while it is possible to estimate the depth of cancer from the result of occult blood test in the feces alone it may be difficult to estimate or identify the hemorrhaging site (that is, the pathological lesion). Although no major differences were found among the reagent kits in terms of their sensitivity for colon and rectal cancer the sensitivity for rectal cancer was higher than for colon cancer although to only a small degree. A major factor to account for this may be the fact that because of the longer retention time in the colon rather than the rectum Hb is decomposed under the influence of the enteral bacteria and enteral enzymes even when hemorrhaging occurs from the cancer lesion[16)].

We have investigated the appropriateness of the cutoff value by comparing sensitivity and specificity. Based on the present results, we have compared the sensitivity and specificity associated with the hypothetical cutoff value taken as the concentration at which the values for [(1 - sensitivity)^2 + (1-specificity)^2] assumed a minimum with the sensitivity and specificity associated with the cutoff values recommended by the manufacturers for the various reagent kits. It was possible to find differences among the reagent kits at the recommended and hypothetical cutoff values. While NS had a roughly 5% lower specificity with the hypothetical cutoff value than with the recommended cutoff value its sensitivity was 4% greater. On the other hand, JIA had a 5% higher specificity while the sensitivity remained as it is. In view of these results, it is clear that the cutoff value too has a significant influence on sensitivity and specificity. Similarly, comparisons were also made with regard to the correct diagnosis rates between the recommended and the hypothetical cutoff values. Some of the reagent kits yielded higher correct diagnosis rates with the hypothetical cutoff value while some other kits did not give rise to any significant changes in the correct diagnosis rates between the hypothetical and recommended cutoff values. In view of this, the use of the hypothetical cutoff value may make it possible to increase sensitivity and/or specificity beyond the present level while maintaining the correct diagnosis rate at the level achieved with the recommended cutoff value. Given the fact that the use of the hypothetical cutoff value may result in significant changes in the sensitivity and specificity associated with the recommended cutoff value, it would seem important for each facility to set its own cutoff value in accordance with the particular purpose for which the facility uses the kit.

Since fecal samples submitted in the hospital were used, the present study results may serve as a comparison of the sensitivity and specificity of the different reagent kits in the event that the immunological occult fecal blood test is applied primarily as an indicator for assessing the

pathological condition. Yet, immunological occult fecal blood test is mainly used for screening colorectal cancer, and it is thus necessary to take into account factors such as the accuracy of fecal sampling and the stability of the fecal sample after sampling, that is, factors not considered in this study[17]. In screening tests, the prevalent practice is that the subject takes or sends a fecal sample at home and submits it to the hospital or screening center the following day. The samplers of the reagent kits currently on the market are provided with a preserving solution as a means of ensuring stability after fecal sampling. Hb is known to become inactivated when samples have been kept at a high temperature for a prolonged time after sampling although the different kits show certain differences in degree[17) - 20)]. Allowing for this it seems best to let each facility decide on its own what it considers the most appropriate cutoff value in case of actual screening service operation.

Conclusion

1. Differences in sensitivity and specificity of the four reagent kits used in this study conducted with 85 colorectal cancer patients and 125 normal healthy controls were found.
2. The likely factors that may influence the sensitivity of the reagent kits are the depth of the cancer, the setting of the cutoff value, and the performance of the antibody used by the respective reagents. While the sensitivity for rectal cancer was slightly higher than that for colon cancer there was no major difference between the two.
3. The likely factors that may affect specificity are the influences due to the final decal concentration of each reagent kit and the cutoff value.
4. A comparison was made between the results obtained with the recommended cutoff value and the results obtained with the hypothetical cutoff value. The result shows that some of the kits exhibited differences between the recommended cutoff value and the hypothetical cutoff value. For all of the kits, it seemed possible to improve sensitivity and/or specificity by using the hypothetical cutoff value, without significantly changing the correct diagnosis rate.

The authors wish to conclude this paper by expressing their sincere thanks to all companies concerned for their cooperation in this study.