Studies on "HM-JACK" for Fecal Occult Blood Test Analyzer

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Human hemoglobin
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I. Introduction

Changing dietary patterns have increased the incidence of colorectal cancer. The immunological fecal occult blood test is used at many facilities as a screening procedure to detect invisible traces of lower gastrointestinal bleeding, including bleeding attributable to colorectal cancer. To enable the examination of very large numbers of specimens in the course of mass screening, an automated analyzer has now been developed.

We used the fully automated fecal occult blood test analyzer HM-JACK (Kyowa Medix), conducting a basic investigation of its performance. Here we will report on some of the findings obtained from this basic study of the analyzer.

II. Methods and Materials

1. Reagent

The EXTEL "Hemo-auto" reagent and a dedicated stool sampling container, both of which are used exclusively for the HM-JACK, were employed according to the maker's instructions.

2. Analyzer

The HM-JACK fully-automated fecal blood test analyzer was used according to the maker's instructions.

3. Comparative reagent and analyzer

OC-Hemodia (Eiken) and the OC-sensor [1) 2)] were used as a comparative reagent and analyzer, respectively, according to the maker's instructions.

4. Materials

Stool specimens were collected from outpatients and inpatients and transferred to the clinical laboratory of our center for fecal occult blood testing.

5. Hemoglobin solution

Lyophilized human hemoglobin (Sigma) was weighed out and dissolved in a buffer placed in the HM-JACK stool sampling container to prepare a hemoglobin (Hb) solution.

III. Principle of measurement

HM-JACK is a sophisticated measuring analyzer designed on the basis of the latex agglutination method [3) 4)]. A working curve for the analyzer is calculated based on the master curve, and the master curve is corrected by the standard (at 2 concentrations). The principle of measurement in this case is based on integrated spherical turbidimetry (IST). When a 0.5-mg stool specimen is collected and dissolved in 1.25 ml of buffer in the stool sampling container, the 100 ng/ml buffer solution contains 250 μg/g stool.

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Table 1. Intra-day assay

	Hb solution					Hemoccult positive stool specimen			Stool specimen		
	A	B	C	D	E	A	B	C	A	B	C
1	19.5	68.3	124.3	551.7	1143.3	47.9	170.6	543.6	122.8	166.3	544.1
2	15.7	66.5	121.3	534.6	1107.6	47.1	172.0	553.2	147.3	163.4	606.5
3	18.2	67.9	122.7	550.4	1142.7	48.6	170.9	536.4	141.3	165.1	554.7
4	17.4	68.0	121.1	540.3	1129.1	48.4	172.7	553.9	142.8	161.8	587.5
5	18.3	66.8	119.5	538.0	1110.4	48.3	162.1	554.2	141.4	157.8	582.6
6	18.2	66.0	120.2	530.7	1108.9	46.4	166.1	549.2	135.0	154.2	597.5
7	17.8	67.2	121.9	541.8	1108.3	49.3	166.7	530.6	137.4	157.5	583.5
8	19.2	66.9	120.7	523.1	1116.6	47.1	163.5	559.6	145.5	155.7	603.9
9	18.0	63.9	117.9	527.9	1072.3	44.9	161.7	527.2	124.3	150.4	557.7
10	16.4	63.1	117.3	541.8	1081.6	43.1	167.3	571.9	125.4	149.7	595.5
Mean	17.9	66.5	120.7	538.0	1112.1	47.1	167.4	548.0	136.3	158.2	591.3
SD	1.09	1.64	2.00	8.74	21.87	1.89	4.12	13.74	6.71	5.89	21.87
CV %	6.1	2.5	1.7	1.6	2.0	4.0	2.4	2.5	9.1	3.7	3.8

Unit: ng/ml

IV. Results

1. Intra-day assay

Five concentrations of Hb solution samples were prepared by dilution of the Hb solution with different amounts of the assay buffer; these samples and 3 concentrations of hemoccult positive stool specimens were measured 10 times each. Three kinds of thoroughly stirred stool specimens were collected in 10 separate containers for intra-day assay. The results revealed that intra-assay CV for Hb solution sample A (the lowest concentration) was 6.1%, and 2.5% or less for the rest of the Hb solution samples 4 Hb solution samples. Intra-assay CV for stool specimens A, B, and C were 9.1%, 3.7%, and 3.8%, respectively (Table 1).

2. Inter-day assay

The EXTEL standard reagent was measured at two concentrations (a low concentration of 24 ng/ml and a high concentration of 404 ng/ml) over a 14-day period for inter-day assay. Mean hemoccult values for the low- and high-concentration samples were 24.6 ng/ml and 406.0 ng/ml, respectively. Inter-day CV was 3.3% for the low-concentration sample and 1.4% for the high-concentration sample (Table 2).

3. Minimum detection sensitivity

Samples ranging in concentration from 0 to 25 ng/ml were prepared by diluting the Hb solution with the assay buffer. A blank sample (0 ng/ml) was used as the buffer. Hb solution samples at each concentration were measured 10 times. Based on an analysis of the points where mean +2 SD and +3 SD for the 0 ng/ml sample and mean -2 SD and -3 SD for the different concentration samples did not overlap, the minimum detection sensitivity was found to be 7 ng/ml (17.5 μg/g stool), as shown in Fig. 1.

4. Prozone

To evaluate the "prozone" phenomenon, the Hb solution was diluted with the buffer, and measured values were compared with theoretical values. The "prozone" phenomenon was identified for theoretical values of 1,000 ng/ml or more, but negativity was not detected (Fig. 2).

Table 2. Inter-day assay

	Low	High
1	24.5	404.8
2	25.4	416.5
3	25.6	414.3
4	23.1	401.3
5	25.1	415.0
6	26.0	402.4
7	23.3	411.4
8	23.9	404.4
9	25.0	402.9
10	24.3	401.6
11	24.6	407.7
12	24.9	398.3
13	23.9	401.7
14	24.7	402.0
N	14	14
Mean	24.6	406.0
SD	0.81	5.70
CV %	3.29%	1.40%
MAX	26.0	416.5
MIN	23.1	398.3
Range	2.95	18.25

Unit: ng/ml

5. Linearity

In the analysis of the different dilutions of Hb solution, linearity was detected up to about 1,000 ng/ml (Fig. 3A). Two kinds of hemoccult positive stool specimens (measured values: 976.6 ng/ml and 408.1 ng/ml) showed favorable linearity (Figs. 3B and 3C).

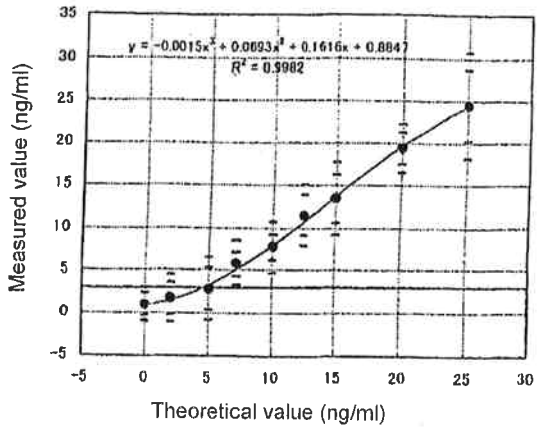

Fig. 1. Minimum detection sensitivity

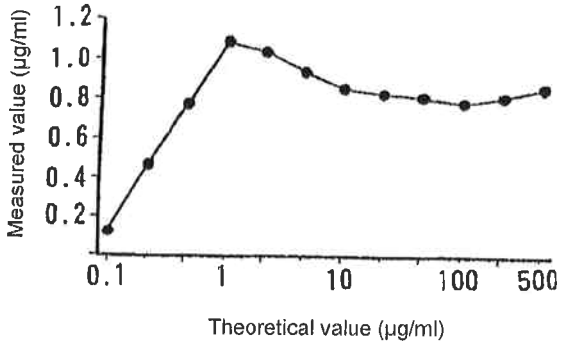

Fig. 2. Prozone

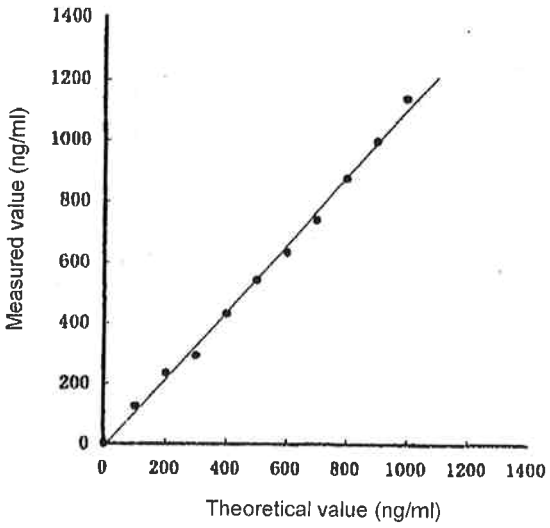

Fig. 3A. Linearity for Hb solution

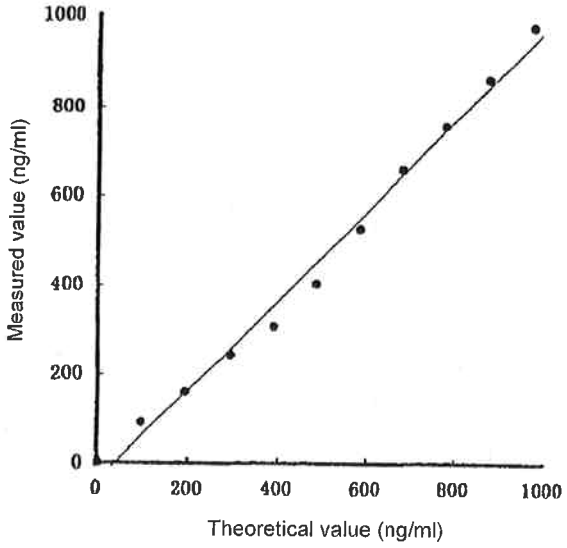

Fig. 3B. Linearity for hemoccult-positive stool specimen

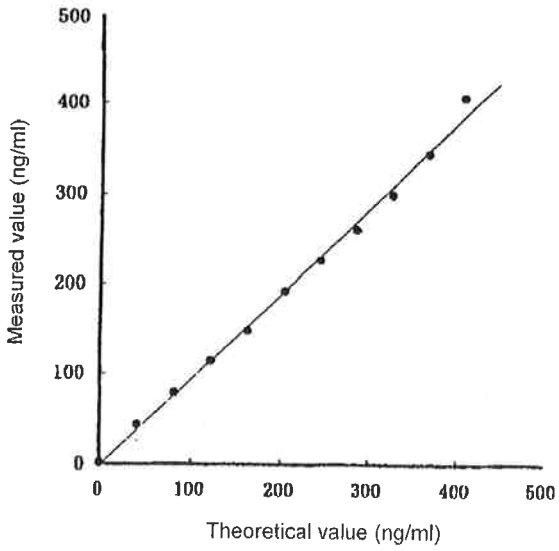

Fig. 3C. Linearity for hemoccult-positive stool specimen

6. Carryover

Hb solution samples ranging in concentration from 500 ng/ml to 500,000 ng/ml were prepared. Five replicated measurements were made of the Hb solution samples, followed by measurements of the buffer (blank sample corresponding to an Hb concentration of zero), to identify the presence or absence of carryover. Carryover was detected in samples at concentrations exceeding 100,000 ng/ml (Table 3).

7. Stability of Hb after stool sampling

Two kinds of hemoccult positive stool specimens (measured values of Hb: 384.5 ng/ml and 118.6 ng/ml, respectively) were collected in several sampling containers and dissolved in the buffer. The dissolved solution samples obtained from each container were stored at 0°C, 4°C, 25°C (room temperature), and 37°C (in an incubator) for 10 days to determine the stability of Hb. Storage of the samples at 0°C and 4°C for up to 10 days did not result

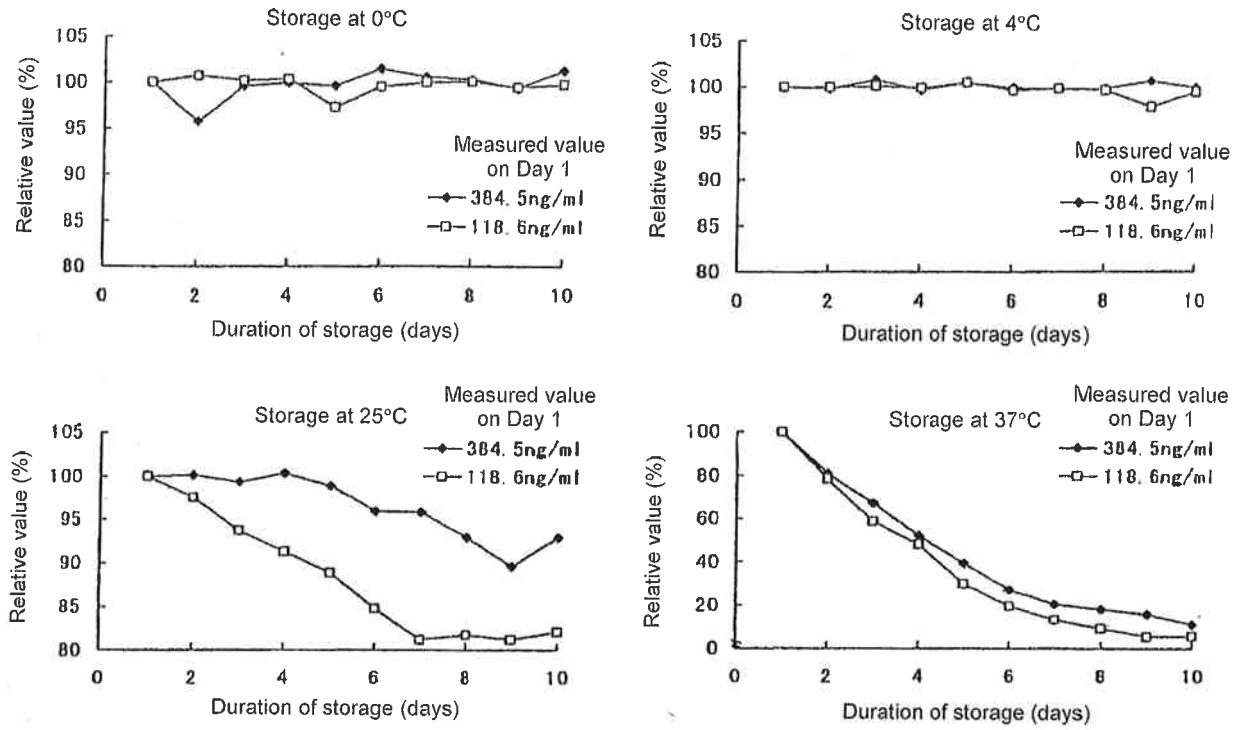

Fig. 4. Stability of Hb after stool sampling

Table 3. Carryover

No.	Concentration of the sample (ng/ml)	Measurement value (ng/ml)	Decision	No.	Concentration of the sample (ng/ml)	Measurement value (ng/ml)	Decision
1	500	565.6		37	20000	825.9	
2	0	0.4	No interference detected	38	0	1.2	No interference detected
3	0	0.6		39	0	0.2	
4	0	0.5		40	0	0.1	
5	0	0.5		41	0	0.4	
6	0	0.3		42	0	0.3	
7	800	890.1		43	50000	811.6	
8	0	0.2	No interference detected	44	0	1.3	No interference detected
9	0	0		45	0	0.2	
10	0	0		46	0	0.1	
11	0	0.4		47	0	0.4	
12	0	0.9		48	0	0.7	
13	1000	1086.8		49	100000	780.1	
14	0	0.4	No interference detected	50	0	13.6	Interference detected
15	0	0		51	0	0.6	No interference detected
16	0	0		52	0	0.3	
17	0	0.5		53	0	0.4	
18	0	0.5		54	0	0.6	
19	2000	1035		55	200000	811.5	
20	0	0	No interference detected	56	0	29.7	Interference detected
21	0	0.3		57	0	0.6	No interference detected
22	0	0.6		58	0	0.2	
23	0	0		59	0	0.6	
24	0	0.2		60	0	0.6	
25	5000	928.8		61	500000	852.7	
26	0	0.4	No interference detected	62	0	97.7	Interference detected
27	0	0.4		63	0	26.4	Interference detected
28	0	0.7		64	0	0.3	No interference detected
29	0	0.2		65	0	0.6	
30	0	0.2		66	0	0.6	
31	10000	851.9					
32	0	0.3	No interference detected				
33	0	0.1					
34	0	0.2					
35	0	0.5					
36	0	0.5					

Table 4. Correlation between HM-JACK and OC-sensor

		HM-JACK		Total
		+	-	
OC-sensor	+	18	0	18
	-	6	186	192
Total		24	186	210

Concordance rate: 97.1%

in a decrease in Hb levels in either stool specimen. Hb level on Day 1 was defined as 100%. When the two specimens were stored at 25°C, this level decreased by approximately 10% for the 384.5 ng/ml specimen and 20% for the 118.6 ng/ml specimen. For both of the samples stored at 37°C, the Hb level decreased by approximately 20% on Day 2 and by approximately 50% on Day 4 (Fig. 4).

8. Correlation

Hb levels obtained by the HM-JACK were compared with those obtained by the OC-sensor in 210 stool specimens. When cutoff values were defined as 12 ng/ml (corresponding to 30 μg/g stool) for the HM-JACK and as 100 ng/ml (corresponding to 20 μg/g stool) for the OC-sensor, the concordance rate was 97.1% (Table 4).

V. Discussion

The immunological method is currently the primary method of fecal occult blood testing. It is widely used as a screenings procedure for colorectal cancer in mass screening and health check-ups.

In this study, its measurement range was found to be sufficient (7 to 1,000 ng/ml). The "prozone" phenomenon was detected in specimens at concentrations exceeding 1,000 ng/ml. However, because such specimens did not become negative, prozone phenomena are unlikely to interfere with measurement. Carryover was observed in samples of 100,000 ng/ml or more, and measured values in these samples were around 800 ng/ml. Thus, caution should be exercised when measured values are around 800 ng/ml. Verifying measured values using IST is important in identifying prozone phenomena and carryover.

Intra-day assay CV was 6.1% or less for Hb solution samples, 4.0% or less for hemoccult positive stool specimens, and 9.1% or less for stool specimens, yielding very satisfactory results. Inter-day CV was also satisfactory, with 3.3% for Hb solution samples and 1.4% for hemoccult positive stool specimens.

The stability of Hb contained in stool sampling containers was very favorable. It had decreased by 20% or less on Day 10, even in samples stored at room temperature. Thus, such stool sampling containers appear to be appropriate for mailing stool specimens for colorectal cancer screening.

The concordance rate between the HM-JACK and the OC-sensor was 97.1%. In 6 patients whose stool specimens were positive for Hb using the HM-JACK but negative using the OC-sensor, the diagnoses were chronic renal failure (1), ulcerative colitis (2), gastric ulcer (1), and unknown (2). The higher rate of positives yielded by the HM-JACK related to the OC-sensor may imply a problem related to stool sampling and differing specificity in latex reagents [3) 4)]. Resolution of this issue must await further studies.

VI. Conclusions

The fully-automated fecal occult blood test analyzer HM-JACK was shown to yield basically favorable intra- and inter-day precision and accuracy, sufficient measurement range, and stability of Hb after stool sampling, among other features. This analyzer is fast and easy to use, analyzing 180 samples/hr. In addition, because stool sampling using the pierced procedure yields very favorable reproducibility, the HM-JACK appears to be a useful testing analyzer in terms of routine practice.

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