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# Evaluation of the Extel "Hemo Auto" HS and the Hemo Auto MC Feces Collection Container Using the HM-JACKarc Fully Automated Fecal Occult Human Hemoglobin Analyzer.

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[Key Words] Fecal occult blood, latex cohesion method, HM-JACKarc

## Introduction

Fecal occult blood testing (testing for human hemoglobin in feces) is generally prevalent as a primary screening method used on digestive tract system patients for diseases such as colon cancer. Initially, a chemical measurement method (stool guaiac test for fecal occult blood – gFOBT) was used, which utilized hemoglobin peroxidase activity. However, because this method could react with animal hemoglobin, which is difficult to distinguish from human hemoglobin, it could easily lead to false positives, so it had the disadvantage of requiring a restricted diet when being used. Therefore, an immunochemical measurement method (fecal immunochemical test (FIT)) that uses anti human hemoglobin antibodies, which are highly selective for human hemoglobin, is now predominantly used. Today, there are a variety of measurement methods that have been adopted by various companies for FIT, using latex agglutination[1)2)], colloidal gold agglutination[3)4)], and immunochromatic[5)] methods as intensification methods for the detection of microscopic bleeding in samples. In all of these methods, improvements are highly sought after in the reproducibility of the approximate cutoff values (30 μg/g of stool (we use 30 ng/ml)) that are used during medical checkups.

However, it is well known how easy it is for the characteristics of hemoglobin to denature and decompose. In particular, due to the influence of temperature and bacteria within the feces, denaturing and decomposition can become the cause of possible misdiagnoses. This makes improvements in hemoglobin stability after collection of the feces highly desirable.

When our company developed the HM-JACKarc fully automated fecal occult human hemoglobin analyzer, we took an extensive second look at the HM-JACK system, and developed a system that combined the Extel "Hemo Auto" HS used as a special reagent, and the "Hemo Auto" MC feces collection container into a single unit.

In particular, we believe that the Extel "Hemo Auto" HS that we use for the latex agglutination method, in order to improve the reproducibility of the approximate cutoff value, has increased reproducibility by increasing the amount of turbidity change (ΔIST value) around the time limit, when measuring the specimen's approximate cutoff value, above conventional values (increasing the sensitivity of the reagent). We then evaluated the lowest detection limit according to the ΔIST value of extremely low value specimens, and the increased sensitivity in reproducibility concurrently and on different days that used low value specimens and we also evaluated the basic performance of the reagent. We also report on the evaluation we made on the stability of hemoglobin after collection of feces for the "Hemo Auto" MC feces collection container.

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**Photograph of the HM-JACKarc fully automated fecal occult human hemoglobin analyzer**

## I. Methodology

### A. Clinical Laboratory Instruments and Reagents

We used an "HM-JACKarc" fully automated fecal occult human hemoglobin analyzer (H: 500 mm x W: 600 mm x D: 610 mm) (see photo), and its specialized "Hemo Auto" MC feces collection container and Extel "Hemo Auto" HS specialized reagent. We also used an "HM-JACK" fully automated fecal occult human hemoglobin analyzer and its Extel "Hemo Auto" HS specialized reagent as a convention method.

### B. Extel "Hemo Auto" HS Measurement Principles

Through an antigen antibody reaction that occurs between the anti-hemoglobin antibodies, which are on the latex granules, and the hemoglobin in the feces, the latex granules cohere through the hemoglobin and cause a change in turbidity. The HM-JACKarc, which has adopted an integrating sphere tubidimetric (IST) in an optical system, calculates the turbidity from the light that penetrates and the previously scattered light, seizing on this latex agglutination. The amount of change within a time limit of this turbidity (the *Δ*IST value) changes according to the hemoglobin concentration in the specimen. With this reagent, based on standard curves that are adjusted with Extel hemoglobin standard HSL and H, the hemoglobin concentration is calculated from each of the *Δ*IST value that has been sought in each specimen.

As opposed to absorbance methods, when making measurements using integrating spherical turbidimetrics highly sensitive measurements become possible, since turbidity can be a perceived event with a small amount of agglutination. Also, since turbidity can be detected even in the high concentration range, the dynamic range can be widened, and it has the advantage that it is difficult for the prozone effect to occur.

### C. Hemoglobin Stability

We dissolved fecal specimens obtained from 3 patients with a buffer solution in feces collection containers, stored them under refrigeration and confirmed hemoglobin stability.

## II. Results

### A. Lowest Detection Sensitivity (Analysis Sensitivity)

Using the 2SD method that takes as the final detection sensitivity the concentration where there is no overlap between average *Δ*IST value for a zero concentration + 2 SD, and the average *Δ*IST value of the standard product in each concentration – 2SD, when we looked for the lowest detection sensitivity, it was 1.25 ng/ml (Fig. 1).

### B. Concurrent Reproducibility

We performed 12 measurements using feces specimens of 3 concentrations of a low value, mid value and high value, and evaluated the concurrent reproducibility. The results were that the CV value was 2.5% at the low value, 1.1% at the mid value, and 1.1% at the high value (Table 1).

### C. Different Day Reproducibility

Using feces specimens of 3 concentrations of a low value, mid value and high value, we performed 5-day measurements over a 9-day period, and evaluated reproducibility over different days. The results were that the CV value was 4.9% at the low value, 4.2% at the mid value, and 2.8% at the high value (Table 2).

**D. Dilution Linearity**

We diluted 2 concentrations of feces specimens in feces collection containers with a buffer solution and evaluated dilution linearity. The results were that we obtained good linearity through the origin (Fig. 2).

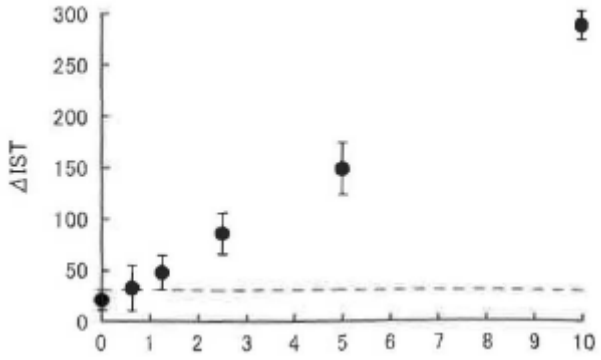


**Fig. 1 – Lowest Detection Sensitivity (Analysis Sensitivity)**

**E. Accretion Recovery**

We accreted preparations at 3 concentrations of hemoglobin in feces specimens, and evaluated the recovery rates. The results were that we obtained 98.3 to 103.7% recovery rates (Table 3).

**Table 1 – Concurrent Reproducibility**

| | Low value | Mid value | High value |
|---|---|---|---|
| N | 12 | 12 | 12 |
| Mean (ng/ml) | 11.8 | 59.6 | 292.2 |
| SD | 0.3 | 0.6 | 3.2 |
| CV% | 2.5 | 1.1 | 1.1 |

**Table 2 – Different Day Reproducibility**

| | Low value | Mid value | High value |
|---|---|---|---|
| N | 5 | 5 | 5 |
| Mean | 11.3 | 56.1 | 279.5 |
| SD | 0.6 | 2.4 | 7.9 |
| CV% | 4.9 | 4.2 | 2.8 |

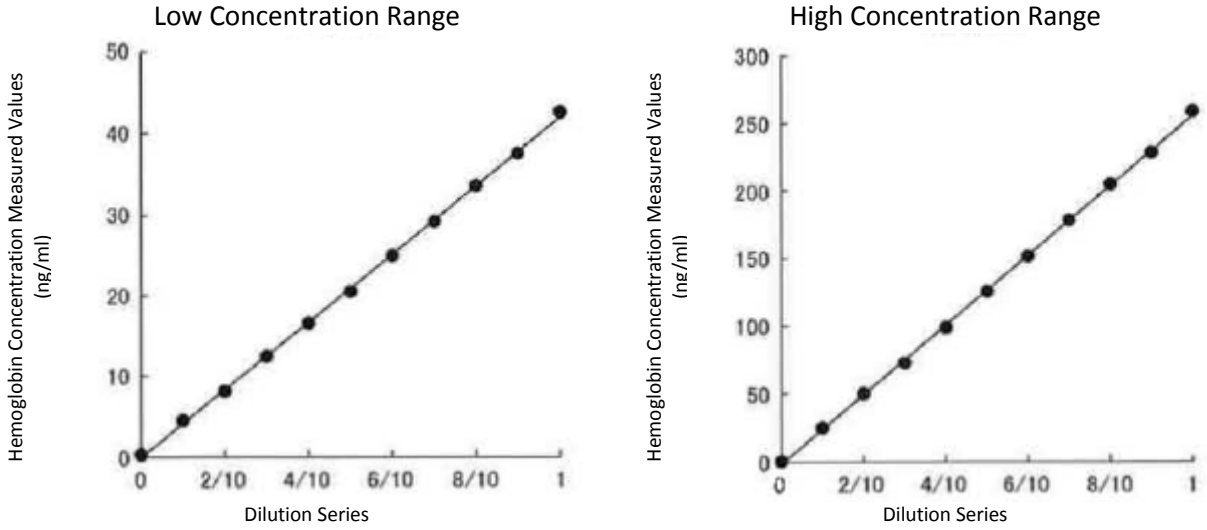


**Fig. 2 – Dilution Linearity**

**Table 3 – Accretion Recovery**

| | Accretion Concentration (ng/ml) | Theoretical Value (pg/ml) | Measured Value (pg/ml) | Recovery Rate (%) |
|---|---|---|---|---|
| Low Value | | - | 10.3 | |
| | 20.5 | 30.8 | 30.3 | 98.3 |
| | 40.1 | 50.4 | 51.1 | 101.3 |
| | 82.3 | 92.6 | 96.0 | 103.7 |
| Mid Value | | - | 51.4 | |
| | 20.5 | 71.9 | 72.5 | 100.8 |
| | 40.1 | 91.5 | 92.9 | 101.5 |
| | 82.3 | 133.6 | 134.9 | 100.9 |
| High Value | | - | 269.9 | |
| | 20.5 | 290.4 | 291.4 | 100.4 |
| | 40.1 | 310.0 | 315.9 | 101.9 |
| | 82.3 | 352.1 | 360.0 | 102.2 |

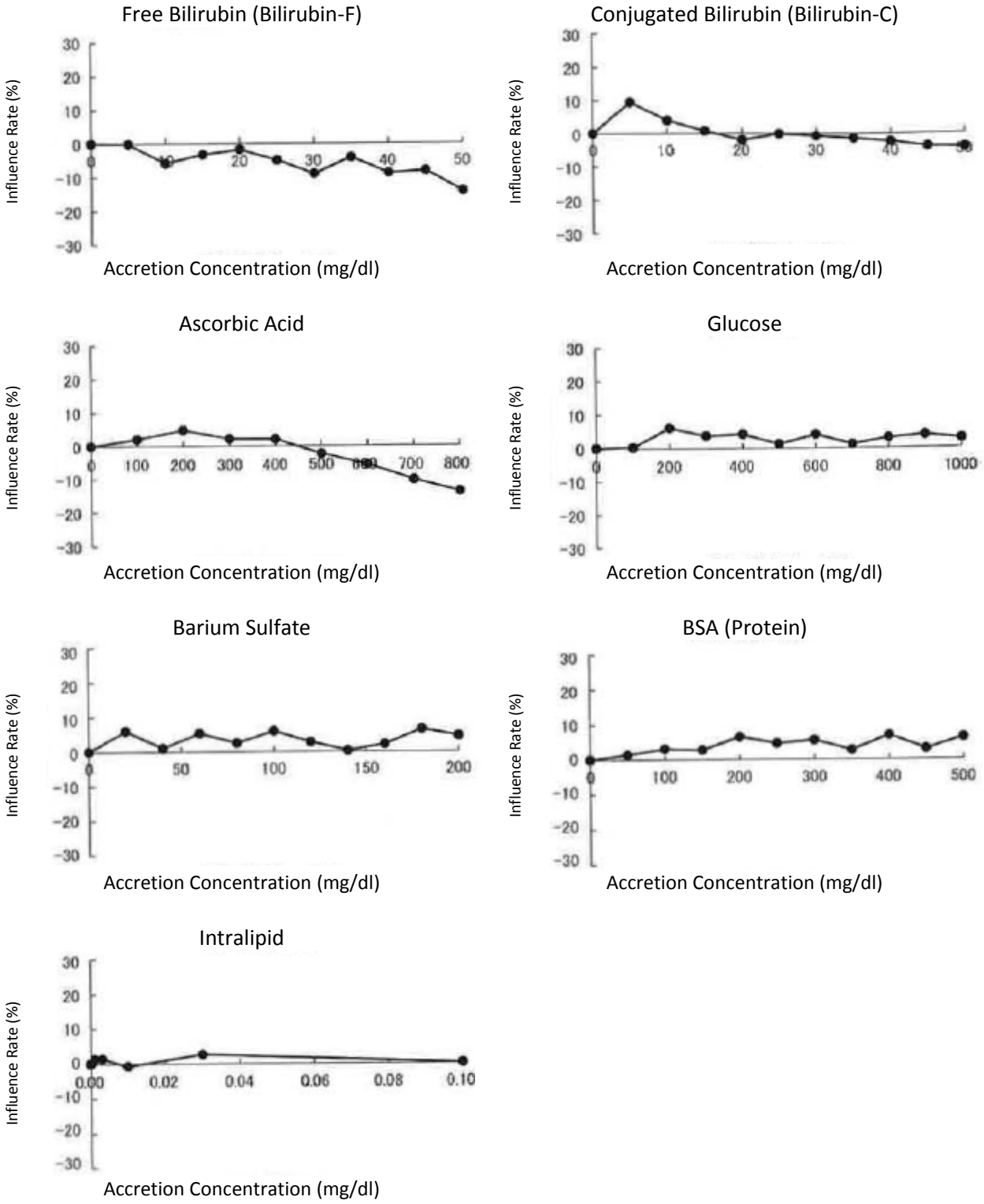


**Fig. 3 – Influence of Coexisting Materials**

**F. Influence of Coexisting Materials**

We added free bilirubin, conjugated bilirubin, ascorbic acid, glucose, barium sulfate, BSA (protein), and Intralipid to the standard product, and evaluated the influence of coexisting materials. The results were influence rates of within ± 10% of measured values, with free bilirubin at 50 mg/dl, conjugated bilirubin at 50 mg/ml, ascorbic acid at 600 mg/dl, glucose at 1000 mg/dl, barium sulfate at 200 mg/dl, BSA (protein) at 500 mg/dl and Intralipid at up to 0.1%.

**G. Prozone Effect**

We prepared a high concentration solution of hemoglobin using the standard product, and evaluated whether or not there was a prozone effect. The results were that at up to approximately 2000 ng/ml the prozone effect was not observed, and that up to approximately 200000 ng/ml, the measured value did not fall below the 400 ng/ml that is the upper limit of the measurement range (Fig. 4)

**H. Correlation with Other Systems**

We evaluated the correlations that were measured for this reagent and out Extel "Hemo Auto" with both the HM-JACKarc and the HM-JACK, using 35 specimens. We obtained results of a regression y=0.96 x+0.28, and a correlation coefficient r=0.996 (Fig. 5).

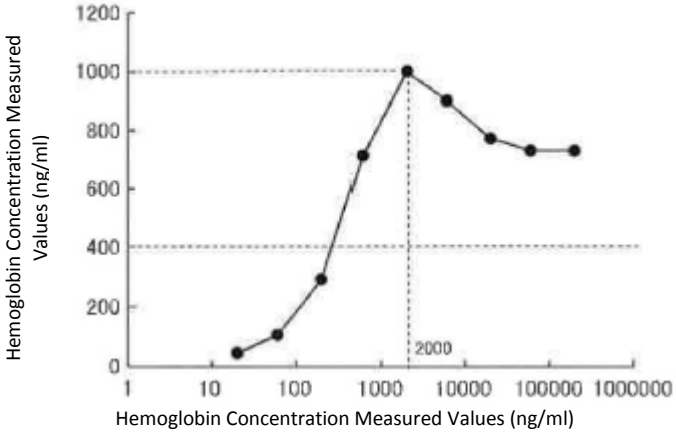


**Fig. 4 – Prozone Effect**

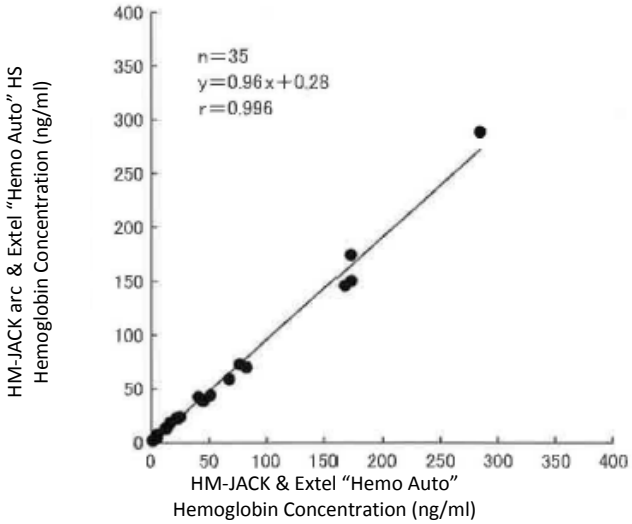


**Fig. 5 – Correlation with other methods**

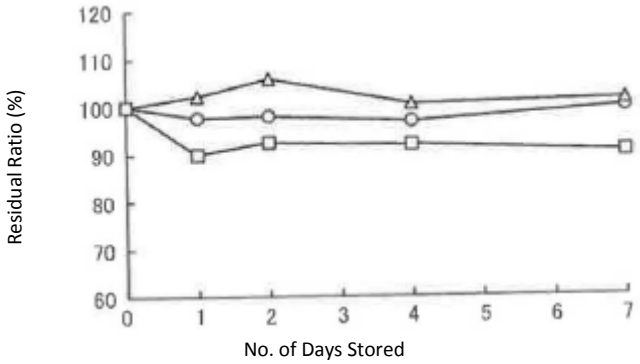


**Fig. 6 – Storage Stability of Specimens**
(○: Specimen A, Δ: Specimen B, □: Specimen C)

**I. Stability of Hemoglobin in a Feces Collection Container Buffer Solution**

Although the stability varied by feces specimen, on the 4th day, the residual ratio averaged 96.4%, and on the 7th day it was 97.1% (Fig. 6).

**III. Conclusion**

We have developed the HM-JACKarc fully automated fully automated fecal occult human hemoglobin analyzer, and have developed it as a single unit with the Extel "Hemo Auto" HS specialized reagent and the "Hemo Auto" MC specialized feces collection container. And we achieved high sensitivity for Extel "Hemo Auto" HS and were able to clearly demonstrate good hemoglobin stability for the "Hemo Auto" MC feces collection container.

Screenings for colon cancer include chemical method fecal occult blood tests, immunochemical method fecal occult blood tests, sigmoid colonoscopies, endoscopic examinations, X-ray examinations and digital rectal examination. Among these, only the fecal occult blood examinations are indicated as the recommended Grade A by the Ministry of Health, Welfare and Labor's Colon Cancer Screening Research Committee. Furthermore, using the immunochemical method for these tests is more desirable than the chemical methods because of their excellent sensitivity and having the advantage of not requiring a restricted diet.

Up until now, we have been selling the Extel "Hemo Auto" series, which is an immunochemical method based fecal occult blood test[1)]. For the Extel "Hemo Auto" HS that we reported on in this article, the lowest detection sensitivity (analysis sensitivity) was highly sensitive when compared to theoretical measurement methods[2)] based on conventional latex agglutination. Therefore, this improves the reproducibility of measured values at the approximate cutoff value, and makes even more accurate measurements possible.

Since the amount of feces collected is made smaller, due to this increased sensitivity, we have been able to minimize the mixing of bacteria with the feces, which is one of the factors that causes hemoglobin instability in the feces collection container[6)], and as a result we believe that this has contributed to an improvement of hemoglobin stability after feces collection. In fact, we have been able to obtain good results after one week of cold storage for hemoglobin stability in a feces collection container buffer solution. And in this way we have been able to reduce the risk of a deterioration of measured values due to hemoglobin instability after the collection of the feces.

By using the "Hemo Auto" MC feces collection container to reduce the risk of a deterioration in measured values due to a change in the stability of the hemoglobin within the feces, and by improving reproducibility of measured values at the approximate cutoff value, due to the increased sensitivity of the Extel "Hemo Auto" HS, we can anticipate the ability to obtain more accurate measured values in colon cancer screening tests.

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