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Novel artificial stool material for external quality assurance (EQA) on a fecal immunochemical test for hemoglobin (FIT): The confirmed utility of stable hemoglobin and an internal standard material

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ABSTRACT

The fecal immunochemical test for hemoglobin (FIT), which detects lower gastrointestinal bleeding, is widely accepted for population-based colorectal cancer (CRC) screening programs. However, the FIT screening process has not been standardized yet, and standardizing the pre-analytical phase and establishing an external quality assurance (EQA) program compliant with ISO requirements is urgently needed. Although there have been various attempts to establish EQA materials suitable for FIT, no materials have yet been reported to have sufficient uniformity and acceptable immunochemical stability of hemoglobin (Hb). The Health Care Technology Foundation (HECTEF; Tokyo Japan) is now developing a ready-to-use artificial stool containing Hb and an internal standard, glycerol. Accordingly, we verified the adaptability and efficacy of this material for the evaluation of the specimen collection phase of FIT. This material uniformly contained both Hb and glycerol. The glycerol allowed us to estimate the weight of the collected artificial stool and to correct the Hb concentration with the estimated weight. Furthermore, the stability of both Hb and glycerol were confirmed to be sufficient for an EQA material under appropriate storage, in-use, repeated freeze-thaw, and heated conditions. These in-house performance characteristics suggest that HECTEF artificial stool is acceptable as an EQA material for FIT.

1. Introduction

Fecal occult blood test (FOBT) detects lower gastrointestinal bleeding caused by cancer, adenoma, and/or polyps. FOBT is the most studied and the most frequently used tool for colorectal cancer (CRC) screening. In FOBT, fecal hemoglobin (Hb) is measured by two different measurement principles: the guaiac-based method (gFOBT) and the immunochemical method (fecal immunochemical test for hemoglobin [FIT]).

With regard to gFOBT, Hb is measured based on the pseudoperoxidase activity of heme. The epidemiological efficacy of gFOBT screening is so significant that the mortality and incidence of CRC were decreased in randomised clinical trials [1,2]. However, gFOBT has performance issues, such as interference with food and drug, low sensitivity, and poor quantitativity as well as technical issues, such as difficulties in automation and low throughput [3].

FIT overcame those defects and has since been recommended for CRC screenings [4,5]. Because quantitative FIT allows us to control the positivity rate by choosing an optimum cut-off value for Hb according to the limitations of the resources available to gastroenterologists and endoscopes, the quantitative FIT is widely used in population-based CRC screening programs.

Although FIT is considered to be a reference method of CRC screening, with > 40 brands of quantitative and qualitative FIT kits commercially available, FIT screening processes have not yet been standardized [6,7]. Therefore, establishing a standardized screening process compliant with ISO15189, which specifies requirements for the quality and competence in medical laboratories, and ISO17043, which specifies general requirements for the competence of providers of proficiency testing schemes and for the development and operation of proficiency testing schemes, is urgently required.

In order to improve this situation, in Japan, where the quantitative

Abbreviations: CRC, colorectal cancer; FOBT, fecal occult blood test; FIT, fecal immunochemical test for hemoglobin; gFOBT, guaiac-based FOBT; EQA, external quality assurance; Hb, hemoglobin

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FIT became popular earlier than in other countries, the establishment of both FIT standard materials and external quality assurance (EQA) materials has been investigated since the 1990s. In 2012 and 2015, the use of the unit "μg Hb/g feces" or "μg Hb/mL feces" were proposed in order to compare the test results obtained from different manufacturers' FIT systems [8,9]. Furthermore, in 2017, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a working group to draft a guideline for FIT standardization. The two main tasks of the working group are standardizing the pre-analytical phase and establishing EQA and third party internal quality control (IQC) programs, as indicated in its terms of reference [10]. These descriptions reflect the urgent and strong need to establish appropriate EQA materials for FIT.

EQA materials for FIT ought to have the following performance characteristics: physical characteristics mimicking those of natural human stool, adequately uniform and stable Hb in the material, and ready-to-use property with minimized errors of preparation. In addition, in order to implement better surveillance and analyses, an appropriate internal standard would allow us to estimate the weight of the material collected and to perform weight correction of the Hb concentration.

The Health Care Technology Foundation (HECTEF; Tokyo Japan) is now developing an artificial stool material that fully satisfies the above criteria. This material is made from rice powder and includes glycerol as an internal standard. In the article reported by Miike et al. [11], Hb was measured only by gFOBT, and the adaptability for FIT was still unknown. Accordingly, the HECTEF kindly offered us the prototype material, which has better Hb stability than the one reported in the last article, and we verified whether or not the material is suitable as an EQA material for FIT.

2. Materials and methods

2.1. HECTEF artificial stool

The prototypes of the artificial stool were kindly supplied by HECTEF. They consisted of two different Hb concentrated artificial stool materials: LOW and HIGH materials, containing 59 and 119 μgHb/g stool, respectively. Those Hb concentrations were determined by HECTEF using an OC SENSOR DIANA system (Eiken Chemical Co., Ltd.) by preparing the artificial stool extracts according to the weighing method described below, in Section 2.2. Approximately 2 to 3 g of the material was dispensed in separate brown glass bottles and sealed with a rubber stopper and a screw cap. The vials were kept at −20 °C until the tests. Before using the material, the vials were kept at 25 °C for at least 20 min in order to thaw the material completely, and the material was mixed well using a spatula.

2.2. Preparation of the artificial stool extracts

The extracts for Hb and glycerol measurement were prepared in two different ways: by the weighing method and by the sampling bottle collection method.

For the weighing method, the weighed artificial stool was collected in a glass tube and dispersed completely by vortex in 4 mL of the buffer obtained from OC-Auto Sampling Bottles (Eiken Chemical Co., Ltd., Tokyo, Japan). After centrifugation for 5 min at 2000 ×*g*, the supernatant was obtained.

For the sampling bottle collection method, the OC-Auto Sampling Bottle was used in 2 different ways: following and not following the instructions. For the "following instructions" attempt, the material was collected in accordance with the instruction manual with two modifications: First, in order to avoid contamination of the buffer into the artificial stool, the sample probe was wiped with a facial tissue before the specimen collection. Then, just before inserting the sample probe into the bottle, the pointed end was rubbed with a facial tissue in order to remove the extra artificial stool on the pointed end of the probe [12]. For the "not following instructions" attempt, the material was collected intentionally in erroneous ways, e.g. too much material was collected by putting the material on the pointed end of the probe excessively, or too little was collected by covering only a part of the groove on the sample probe with the material.

After the specimen collection, the sample collection device was shaken vigorously, and two layers of aluminum seal were pierced using a pipette tip. Lastly, the bottle was squeezed using a pair of pliers, and the filtrate was taken into a sample cup using a micro pipette.

2.3. Measurement of Hb and glycerol

Hb was measured using an OC SENSOR DIANA system according to the manufacturer's instruction. Because glycerol can be quantified using commercially available triglyceride reagents [10], glycerol was measured using H7170S (Hitachi High-Technologies Corporation, Tokyo, Japan), Ekdia XL 'Eiken' TG II (Eiken Chemical Co., Ltd.), and Lipid Calibrator XL 'Eiken' (Eiken Chemical Co., Ltd.). The R1 and R3 of the triglyceride reagents were mixed at a ratio of 3 to 1, and the mixture was set in the R1 rack of the apparatus. Two microliters of the sample and 257 μL of the triglyceride reagent mixture were mixed, and the absorbance of 800/600 nm at turn 18 was measured.

2.4. Confirmation of Hb and glycerol uniformity

The consistency of Hb and glycerol in the artificial stool were tested according to the "Uniformity of dosage units" section of the pharmacopoeias [13–15]. In brief, 10 extracts were prepared from 10 vials of artificial stool by the weighing method and measured. The acceptance values ($AV = |M - \overline{X}| + ks$) were calculated and verified to meet the acceptance criteria. For the claim values of Hb, we used the values indicated on the labels, while for those of glycerol, we regarded the mean value of 10 measurements as 100.0% because the glycerol concentration was not claimed.

2.5. Correlation between the weight of the artificial stool and the glycerol concentration

In a range of 10 to 50 mg, 7 samples of the artificial stool were collected by the weighing method from a vial, and the glycerol in the extract was measured.

2.6. Long-term stability test of the frozen artificial stool

Three vials each of the LOW and HIGH material were used. On the initial day, the artificial stool was collected from a vial five times by the weighing method and measured. After 135 and 189 days, the extracts were prepared from a new vial and measured in the same way.

2.7. Freeze-thaw stability test

Five vials each of the LOW and HIGH material were used. In a freeze-thaw cycle, the vials were held at 25 °C for 20 min and at −20 °C for 12 h or longer. This cycle was repeated 1 to 4 times to obtain the samples. Three extracts of the artificial stool were prepared by the weighing method and measured, including the reference vials without any additional freeze-thawing.

2.8. In-use stability test

Two vials each of the LOW and HIGH material were used. As the initial samples, three extracts were prepared by the weighing method and measured. After the initial specimen collection, the vials were capped tightly and kept at 4 °C or 25 °C for 6 h. After storage, the artificial stool was collected and measured again in the same way. The p